Age at Death and Gender Determination of Kuwaiti Individuals from Dentine

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Declaration

I declare here that the work for this Thesis was carried out by myself or under my direct supervision.

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Date..30/09/01.....

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Abbreviations

TE	Trace element
Zn	Zinc
Pb	Lead
Mn	Manganese
Fe	Iron
Sr	Strontium
Mg	Magnesium
Cu	Copper
NaOCL	Sodium hypochlorite
NaCL	Sodium chloride
Hcl	Hydrochloric acid
HNO_3	Nitric acid
SO_4	Sulfate
HCO_3	Hydrogen carbonate
CO_2	Carbon dioxide
NO_3	Nitrate
Ln	Logarithm
Sig	Significant
NS	Not Significant
*	Significant at level 0.05
**	Significant at level 0.001

ABSTRACT

The aim of this research was :

1) To develop an accurate method of age estimation of dead Kuwaiti from their teeth based upon the conversion of L- to D- aspartic acid using dentine from the middle third of a permanent tooth.
2) To estimate age from buried teeth and study the influence of temperature , humidity and pH upon the rate of racemization of aspartic acid in dentine buried in the desert.
3) To investigate a possible association between trace element (Zn , Pb , Fe , Mn, Mg, Sr, and Cu) accumulation in dentine and age in Kuwaitis
4) To investigate the possibility of gender determination from the results of racemization and trace element experiments.

Results and Conclusions.

Estimation of age at death from dentine from upper first premolar teeth using High Performance Liquid Chromatography (HPLC) for the separation of D- and L- forms of aspartic acid in minute quantities of dentine has been successfully established in Kuwait and is sensitive enough for practical use. The method was shown to be accurate to +/- 1.2 years.

The affect upon racemization of aspartic acid in teeth buried at depths of 0.5M and 2.0M over a 10 month period demonstrated that in desert conditions in the Middle East racemization continued providing results less constant than in teeth not buried. A standard error of +/- 2.3 years was obtained for teeth buried at 0.5M and +/- 3.0 years at 2.0M. The D/L ration was significantly different between buried and not buried teeth. These results demonstrated continued postmortem racemization in the warm soil and the modification of the racemization rate is discussed in the context of prolonged exposure to body temperature , relatively high pH and low humidity. The findings are important since estimation of the age of an unknown body recovered from the desert must now take into consideration temperature , pH , soil humidity at

the burial depth (as measured at the site) and also the length of time for which the teeth may have been buried.
Further studies now require to be undertaken in conditions where the soil and climate are very different in order that the results of racemization estimates world wide can be interpreted accurately.

The trace element experiments while demonstrating an accumulation of most elements with time did not provide reliable information about the age of the subject at the time of death. The variables and problems concerned are discussed in detail.

Although through using both racemization and trace element methods it was possible to indicate a probability of gender in any tooth examined the degree of accuracy proved to be poor and it is anticipated that DNA analysis will be much more useful. The future role for tooth pulps in DNA identification is briefly discussed.

CONTENTS

Chapter 1

INTRODUCTION AND OBJECTIVES

For the purposes of identification of an unknown corpse, the age at death of a deceased individual is of great importance. For children and adolescents a fairly accurate age determination is possible by assessing the incomplete process of growth and development within the skeleton and the teeth [1]. Age determination in adults may also be accomplished through special consideration of the skeletal system and the teeth. However, age determination is more difficult in adults than children or adolescents [1]. Following completion of the period of growth, age-related changes used in age determination are influenced not only by the age of the individual, but also by numerous endogenous and exogenous factors, such as disease, nutrition and physical strain [1]. It is for this reason that the biological age may deviate considerably from the chronological age, the latter being of interest in forensic practice [1].

Accurate identification of human remains is important for legal, administrative, emotional and ethical reasons. In civil law death changes the civil and juridical status not only of the deceased but also of the relatives for whom also there may be major financial implications. In criminal law failure to identify remains complicates murder cases and may permit one individual to attempt to adopt another's identity [2].

The total number of Kuwaiti POW's and missing persons after the Iraqi war was 609 (599 men and 10 women) as on August 31, 1993. The total includes prisoners of many ages .Of those detained in Iraq. 415 are aged between 16 and 30, 188 are between 31 and 50 and 22 between 51 and 80 years of age. We know that neither the very young nor the very old can long endure imprisonment under wartime conditions

[3]. Although the vast majority of POW's and missing persons are Kuwaiti nationals, the Iraqis also detained foreigners from 8 Nations who were sympathetic to the Kuwaiti cause; 13 Saudis, 5 Iranians, 4 Egyptians, 4 Syrians, 3 Indians, 3 Lebanese and 1 each from Bahrain, Oman and the Philippines. There are also 26 detainees of unknown nationality from the occupation [4].

It is very reasonable to think that in future the Kuwaiti Forensic Science Department will face problems regarding the identification of individuals. Therefore, there is a need to develop methods of identification which will include accurate methods of age estimation.

Until recently the best method of estimating age from teeth has been Gustafson's method with modifications [5 - 12]. However in recent years a new , more accurate , method for estimating age based upon racemization of aspartic acid in enamel and dentine has been developed [13 - 15] **but has not yet been applied to teeth from people living in the Middle East nor have the effects of burial in hot ground been evaluated.**

Accumulation of trace elements with time has been suggested as a means of estimating age but the amount of published data is meager. It is not known whether or not there is any association between rates of racemization or accumulation of trace elements and gender.

The aim of this research was:

1) to develop an accurate method of age estimation for dead Kuwaiti from their teeth based upon the conversion of L- *to* D- aspartic acid using dentine from the middle third of the maxillary first premolar tooth.
2) to study the influence of temperature upon the rate of racemization of aspartic acid in dentine buried for up to 10 months in the desert.
3) to investigate a possible association between trace element (Zn , Pb , Fe , Mn , Mg , Sr and Cu) levels in dentine and age in Kuwaiti individuals.
4) to investigate the possibility of gender determination from the results of the racemization and trace element experiments.

Chapter 2

LITERATURE REVIEW

Forensic Odontology is a sub-specialty of dentistry which may be described as "the proper handling and examination of dental evidence in the interests of justice so that the dental findings may be properly presented and evaluated." [16].

Identifying people by their teeth is not a recent development. Agrippina in AD 49 slew Lollia Paulina because she was perceived as seeking to become the wife of Claudius and this could have prevented Agrippinas' son , Nero , becoming Emperor. When the head of Paulina was brought to Agrippina she was uncertain of its identity so "she opened the mouth with her own hands and inspected the teeth which had certain peculiarities." [17].

With the emergence of craft apprenticeships , in the Middle Ages in the United Kingdom , young men were bound to a Master , and being unable to write the agreement was "signed" by the apprentice by biting into a piece of wax and upon so doing he became "indentured."

Identification has become a Human Right. The United Nations Universal Declaration of Human Rights (1948) [18] , states that ; "Everyone has the right to recognition everywhere as a person before the Law." In the context of Identification the United Nations Member States interpret this as meaning that identification of the dead must be carried out whenever possible.

Forensic dental identification may be described under two headings ; Comparison and Reconstruction.

The work described in this thesis relates to "Reconstruction" and therefore only a very brief account of "Comparative" methodology is included.

2.1 Comparison

Those procedures whereby a forensic dentist compares clinical or postmortem findings with previous clinical records , chartings and radiographs with a view to establishing an identity. Recent developments in DNA profiling provide an important extension of comparative techniques.

The adult dentition comprises 32 teeth each having 5 surfaces. The combinations possible of teeth present , missing teeth , filled surfaces , filling materials and dentures plus those features identifiable on radiographs such as pulp canals , the shape , number and size of roots , as well as the morphology of the supporting alveolar bone is astronomical to the extent that the concept of no two dentitions being alike is a basic tenant of dental identification [19].

A distinct advantage of dental identification over fingerprints is the relatively comprehensive nature of the antemortem data base. An extremely high percentage of the general population in developed countries have visited a dentist at some time in their life [20]. Dentists routinely create dental records for these patients which are often retained for a long period of time. Comparison between clinical or postmortem findings and the dental records of a suspected victim should confirm or exclude an identification.

However several problems related to this method of identification may arise:

1. Illegible records. In many cases dental records are handwritten and determining what treatment has been carried out may be difficult [20] .
2. Lack of adequate detail. Many dentists do not record pre-existing restorations in their chartings [20].
3. Inadequate dental radiographs due to poor quality , poor orientation or the absence of a date [20].
4. Lack of uniformity of charting [20].

5. Human errors in charting such as first premolar instead of second , second molar instead of third and transposition of left for right [20].

In the National Health Service in the United Kingdom dental practitioners are not required to make a detailed chart of the position and shape of existing fillings although existing fillings do have to be noted if further treatment is required for that tooth. The maximum period for which a practitioner is required to retain a chart is four years in Scotland and 18 months in the rest of the UK [20].

The three most common dental charting systems are the Universal system , Palmer's notation and the F.D.I. two digit System. The Universal system is one of the most popular methods of charting in the USA. According to this system each tooth is given one number which is 1 through 16 for the upper jaw and 17 through 32 for the lower numbered in a clockwise direction looking at the patient.
Palmer's notation combines letters and numbers so that upper right is UR and lower left LL. The teeth are numbered from the midline to the third molar , 1-8. Therefore UL4 is the upper left first bicuspid. This is a very simple system to use electronically.

The F.D.I. (Federation Dentaire Internationale) two-digit system is similar to the Palmer . notation but substitutes numbers for the letters. The quadrants are numbered Upper Right 1 , Upper Left 2 Lower Left 3 and Lower Right 4. Therefore the upper left premolar is recorded as 24 and stated as "two-four" and not "twenty four". This system is becoming very widely used and is the system preferred by most forensic dentists. It is interesting to note that discussion of charting systems is one of the most published topics in the field of forensic dentistry [21].

In practice it does not matter *which* system a dentist use provided he uses it *accurately and fully*. Provided all available data is recorded the investigating forensic dentist can readily transpose the charting to the system he is using and an easy comparison is then possible.

2.2 Reconstruction

Reconstruction is the term applied to those procedures whereby forensic pathologists, physical anthropologists and forensic dentists combine their skill and knowledge to "reconstruct" human remains , or fragments of remains .

A dental contribution may be appropriate in attempting to establish the height of the deceased and to a lesser extent make suggestions concerning ethnic origin and gender.

In the fetus, for example, there is a mathematical relationship between mandibular length and total body length. The mandibular length in millimeters is equal to the total crown-heel body length in centimeters while the maxilla is one twentieth the length of the body [22].

Determination of ethnic origin from dental remains is not reliable since the overlap between racial characteristics is so considerable that , realistically , they are of little value in determining the ethnic origin of any single set of remains. A recent very comprehensive account of sex and ethnic characteristics forcibly makes the case for tendencies rather than pathognomonic features . Burris and Harris 1998 [23] have carried out measurements of the width and depth of the palate in an attempt to predict both race and sex. In a sample of 332 living subjects with permanent dentitions measurements were made between cusp tips. Blacks with a more square palate were distinguished from whites primarily by a greater inter-premolar width and first premolar to second molar depth. Simultaneous prediction of race and sex had a correct classification of 48% (about twice that expected by chance). Pooling the two sexes increased the correct classification of race to 83%. Therefore , while useful , the method could not be applied on its own as a method of ethnic identification of unknown remains. A highly detailed and robust scientific study of odontometrics is contained in "Human Adult Odontometrics" by J.A. Kiesser (1990) [24].

Age determination however, which is an extremely important part of "reconstruction" intended to achieve an identification of human remains , is most

accurately achieved by analysis of the teeth and is therefore the most important single contribution that can be made by a forensic dentist. The importance of dental analysis is further appreciated when it is realized that teeth are extremely stable being highly resistant to trauma , fire , water and excessively low temperatures [25]

Before discussing age determination from teeth in detail it is necessary to make some more general observations.

One of the structures that is preserved for a long time after the death of an individual is the tooth. Age related changes in teeth are unavoidable and occur as a part of the normal physiological process of life. In addition, teeth are resistant to destruction by fire. Rarely temperatures around the teeth may exceed 1000 F and the teeth are protected by the soft tissues of the cheeks and the lips which must be destroyed first. The roots of the teeth are encased in alveolar bone, providing an additional layer of protection. In addition to the teeth, the materials used for dental restorations are also resistant to destruction by the environment, often more than the teeth themselves. Many dental restorative materials are resistant to immersion in water, desiccation and rapid decomposition. Gold alloys, fused porcelain and denture teeth will withstand very high temperatures . Silver amalgam , for example will resist temperatures up to 1600 F [26].

Age estimation from teeth is one of the best methods available for the determination of chronological age of individuals with uncertain birth records. [27 - 30].This coupled with their capacity for intact survival for literally centuries postmortem makes teeth particularly valuable in forensic investigations.

In children, the developing teeth have been used as a good indicator of chronological age. Various published methods employed for age determination in children are based on comparison of radiographic development of teeth with standard charts [7,8].

With increasing age there are changes in the shape and texture of the teeth. With the advancement of age, attrition and abrasion of the teeth results in flattening of the cusps and shorting of the cutting edges due to the partial loss of the enamel. The

fissures of the teeth are flattened and may disappear completely while the root apices tend to become short and rounded.

From about the age of fifty onwards, starting at the root and extending up to the crown, the tooth becomes yellow or yellowish brown in color. This change in color may be due to the changes in the optical property of the tooth due to variations in the composition of the hard substance and /or due to the increase in thickness of dentine and cement with age [31].

Recently a more accurate method has been reported based on the degree of racemization of aspartic acid in dentine . In these studies the uncertainty of the predicted age has been reduced to +/- 2 years at the confidence level 95% [27 , 28 , 29 30, 32].

2.3 The dental hard tissues

The hard substances of the teeth are enamel, dentine and cement. A brief description of each of these hard substances is given below in relation to their use as a tool for identification of age, gender and other factors used for the identification of individuals in forensic sciences.

Enamel . is the hardest substance found in the body. It appears as bluish white in thin-ground sections. Under the light microscope enamel appears as thin rods or prisms that stand upright on the surface of the dentine . The protein content of enamel is secreted by cells called ameloblasts. According to Schroeder,1976 [33], when fully developed, it consists of a cell free mineral structure mainly composed of calcium salts in the form of large appetite crystals not subjected to any physiological metabolism . It also contains a high concentration of phosphorus , which may play a major role in the initiation of enamel calcification . Studies on the mineral phase of enamel show the presence of an increased level of Fe, Sn, Cu , and Pb with age [34]. Histologically no difference has been observed in the structure of enamel taken from young and old individuals [35]. The organic matrix makes up only about 0.5% and amino acid analysis showed that one fourth of the amino acid residue is proline. Therefore, the organic matrix of enamel is different from either keratin or proline.

Although it is known that enamel becomes less permeable and more brittle over the years, age induced changes in the level of trace elements and amino acids of enamel are not understood.

Cement is a mineralized, non-vascular connective tissue. Among all the hard tissues of the tooth, cement has a structure quite similar to the bone. The cement is divided into cellular and acellular cement. Except for a thin layer of cellular cement close to the dentine in the apex and the cervical portion of the cement, the remainder of the cement is acellular. The thickness of the cement increase with age, especially near the end of the root. This increase is in part directly dependent upon age [33].

Some teeth, in some individuals, display a massive overproduction of cement known as hypercementosis, which results in a bulbous, irregularly bulging root. If this occurs in several roots of a multi-rooted tooth, the whole can be united into one mass. Hypercementosis of this type can readily be detected in dental radiographs. The cause is unknown, but is unlikely to be solely related to excessive wear because it also occurs in unerupted teeth. One special case of hypercementosis is associated with Paget's disease of bone where a very irregularly bulging mass of cement may occur, with associated bony changes in the jaws.

Cementicles are small nodular bodies of cement that may develop within the periodontal ligament or the alveolar bone. They are rare, and their cause is unclear. Sometimes they become incorporated into the main cement of the root surface [36].

The bulk of the tooth is made up of dentine, which is made by a layer of odontoblasts that line the pulp cavity of the tooth. It consists of about 80% inorganic and 20% organic matter. Almost 92% of the organic matter is collagen and the inorganic part is in the form of hydroxyapatite crystals. [37].

During aging there is an increased mineralization of the primary dentine. This process is called dentine sclerosis. Dentine sclerosis may be due to an increase in the normal peritubular dentine, formation of rhombohedric crystals of the whitlocite type

and deposition of intratubular dentine. The increased mineralization is accompanied by a change in transparency of dentine due to greater optical homogeneity. Due to the sclerosis of dentine, there is a reduction in the lumina of the dentinal tubules, a decrease in the quantity of peritubular fluid and a reduction of diffusion in the direction of the pulp. These changes may be quantitated for age estimation [35].

2.4 Gustafson's method of age estimation

Gustafson 1947 [9], estimated age from a general impression of secondary dentine deposition, cement thickness and periodontosis. This was so successful that Gustafson 1950 [10] devised a system based on six related factors:

1. Dental attrition.
2. Periodontosis
3. Secondary dentine deposition
4. Cement apposition
5. Root resorption
6. Transparency of root (root dentine sclerosis).

In each ground section, of a lower incisor tooth , the features were scored and the points summed to give an overall score. These scores were highly correlated ($r = 0.98$) with known age for 41 individuals,and a regression formula was derived to estimate age from a points score with a standard error of $\pm$ 3.63 years. Nalbandian and Sognnaes 1960 [38] estimated age against known age. The slope and intercept of the regression line,and the correlation, were very similar to those of Gustafson but with a standard error of $\pm$7.9 years. Maples and Rice 1979 [39] found statistical errors in the original Gustafson paper,and Burns and Maples 1976 [40] revised the regression formula, finding a much larger standard error of $\pm$ 11.28 years when it was tested on an independent sample.Their revised formula was also tested by Lucy et al 1995 [12] who found still more statistical problems, but obtained results comparing favourably with Burns and Maples.

2.5 Trace elements

The trace element composition of dentine shows a correlation with age [41]. A possible advantage of using the trace elements level as a tool in forensic science is that different populations may have a different base line expression of trace elements depending on the geographical location and ethnic groups [42]. This is because trace element levels in teeth may vary between people living in different geographical locations and therefore such differences in the trace element levels might possibly be used to relate an individual to a particular ethnic group or a geographical location.

It has been shown that trace element levels in the tooth change in response to various pathological conditions such as untreated renal failure [43]. Since there are changes in the level of trace elements in certain diseased states, the question arises could there be changes in the level of certain trace elements in males and females due to their hormonal /gender status ? Such a finding might prove useful in gender determination in addition to age determination of an unknown body

2.6 Dentinogenesis

Dentine formation (dentinogenesis) takes place in a two-phased sequence; the odontoblast first forms a non-mineralized layer of organic matrix, the so-called predentine or odontogenic zone, which is followed by the formation of the inorganic phase (mineralization) at the so-called mineralization front. The second phase, mineralization, does not initiate until a fairly wide band (10-30 μm) of predentine has been secreted. This layer of unmineralized matrix remains throughout life between the fully formed mineralized dentine and the odontoblasts. The protein composition of the predentine matrix differs from that of mineralized dentine as some protein components are secreted just in advance of the mineralization front. The predentine does not contain any forms of phosphoproteins or Gla-proteins [44].

2.7 Dentine collagen.

Collagen is the major organic constituent of dentine, it represents more than 90% of the organic material [45]. Dentine collagen is almost exclusively made up of type 1 collagen [46] and is known to be highly insoluble, resistant to swelling and

materially stiff in comparison with soft tissue collagen such as that present in skin or tendon [47].

Amino acids in collagen occur in two stereoisomeric forms, designated D- and L-enantiomers. The amino acids are incorporated into the tooth as L-enantiomers. The term racemization describes the conversion of an amino acid enantiomer to its mirror image. The L-amino acids slowly convert to form an equal quantity of the D-isomers, even when they are polymerized into proteins[48].

Hefman and Bada [29] established that during aging a gradual transformation of L-Asp into its D-form (racemization) occurs in dentine from human teeth. This produces a measurable, age-dependent increase in the D-aspartic concentration in the tissue [29]. In general there is a good agreement between estimated and true age, with a 95% confidence interval of between two to six years being achieved. The racemization method has also been succesfully applied to several anthropological problems [49 , 50].However,the ontogenetic age of an individual does not correspond directly with the age of the teeth, since teeth develop gradually and different types of teeth develop at different ages.The synthesis of crown dentine in permanent teeth requires a period of approximately four to seven years.In this process,dentine is built up in layers from the outside moving inwards.The dentine of the first (outermost) layer is therefore several years older than that of the last (innermost) layer.

2.8 The location of examined dentine samples

Ohtani and Yamamoto [48] prepared longitudinal sections from left and right upper central incisors from the same person, and compared D/L ratios between the labial and the lingual sides of the crown. The results showed a tendency for the lingual side to yield a higher D/L ratio [48]. This may be attributed to the fact that the labial side is susceptible to external influence and consequently the environmental temperature which tends to be lower [48].

2.9 Disease and Disorders

Factors such as disease and nutrition may influence racemization kinetics.Obviously, carious dentin cannot be used for racemization analysis. Likewise,some pathological conditions such as dentinogenesis imperfecta may influence protein composition of dentine and consequently affect D/L ratios.

2.10 Post Mortem Environment

The measured D/L ratio in a tooth from skeletonized material is the sum of in vivo and post mortem racemization. Hence the accuracy of the technique depends on minimizing the post mortem effect. Recent demise or a cold post mortem environment may be necessary to avoid significant post mortem racemization.

Ogino *et al* [51] assessed the age-at-death (ontogenetic age) of 14 unidentified cadavers by the use of Asp racemization in dentine. All of the cadavers were later identified,revealing their actual ages.According to Ogino et al [51] *"no significant information can be determined from the discrepancy between the estimated and actual ages and such factors as the cause of death or the amount of time that has elapsed since death"* . In all cases, discrepancies between estimated and actual age were less than $\pm$ 3 years [51].

Likewise, Masters [13] analysed Asp racemization in human dentine in six autopsy cases, where independent age information was available. These cases represented a range of postmortem fates including recent demise, burial and ground surface exposure. Asp age-at-death estimates were , in five of the six cases, within the error of known dental age. In one case, however, the racemization age was inflated (+ 10 years). This sample came from a partly skeletonized corpse. The body had been lying exposed on the ground for a period of 51 days in February and March (San Diego,California, USA), indicating that environmental factors associated with ground surface exposure such as direct sunlight (radiant heat) may increase the rate of postmortem racemization [13]. If a cadaver is stored at outdoor temperature or cooler, the postmortem rate of racemization is expected to be considerably lower than the in vivo rate. Kinetic calculations indicate that post mortem preservation for

5 years at 20°C results in an error of less than 0.3 years in the age-estimation by Asp racemization.

2.11 Coronal and Root Dentin

Results presented by Ohtani and Yamamoto [30 , 52] display different rates of Asp racemization in coronal and root dentin from the same tooth. The extent of racemization tended to be higher in root dentine compared to coronal dentine ; a result confirming the findings of Ogino et al two years previously [51]. The following explanations may be proposed for these findings.

2.12 The existence of a temperature gradient within the tooth

The ambient temperature of crown and root may be different resulting in different racemization rates. It has been shown that there is a heat flow from the periodontal tissue to the tooth surface and a gradient of up to 0.73°C has been reported at 30-31°C ; oral tissue temperature [53]. Breathing, talking, sipping cold or hot drinks etc. could contribute further to thermal differences. The actual magnitudes of such effects are unknown, but it is certain that fluctuations in temperature can bring about significant changes in the rate of racemization.

2.13 The type of tooth examined

Some studies suggest that different teeth have different racemization kinetics. Thus Ogino *et al.* [51] suggested that the separate consideration of individual tooth types may lead to more precise age estimates. However, the number of examined teeth by these authors was not sufficient for a satisfactory analysis. A further advantage of working exclusively with one tooth type is that it is unnecessary to incorporate a tooth developmental correction factor into the calibration data, and the unknown individuals (ontogenetic) age can be estimated directly from the regression line.

The first authors to use Asp racemization for aging were Helfman and Bada for human enamel 1975 [27]. Since then there have been many studies using

racemization in dentine to determine age at death [15 , 30]. The majority of these have been successful in constructing excellent calibration curves which can estimate age in fresh samples to within +/- 3 years, a significant improvement on the traditional morphological methods.

When the technique was used for age determination on archaeological material, it was found that the results deviated from what was expected. Teeth of known age from the Spitalfields Crypt displayed aspartic acid racemization ages, which were too old for young individuals [54]. It was discovered that the teeth contained microorganisms producing enzymes capable of promoting racemization [55], which could have been the cause of the discrepancy. More recent studies have had no more success with Victorian teeth of known age [56], and so it would appear that we are long way from being able to make archaeological age determinations using this technique.

For recently deceased individuals however aspartic acid racemization is a technique, which can offer a significant improvement on the current methods of age determination. Standardization across many laboratories and countries is needed before racemization can be adopted as a reliable technique in the forensic sciences.

2.14 The statistical analysis for age and gender determination

Regression may be defined as the analysis of data to identify the main features of statistical relationships between dependent and independent variables for purposes of description, control, and prediction. The term independent variable (denoted by x) is used. However, epidemiological and other articles may refer to independent variables using any of the following terms; predictor variable, explanatory variable, carrier or classification variable or effect modifier.

Multiple regression is concerned with providing a mathematical model of the linear relationship between a dependent (i.e., outcome variable) and two or more independent or predictor variables. In particular, the mean level of a dependent variable may be predicted from a set of values for the independent variables.

A multiple regression model with two independent variables may be represented as follows:

$Y = B_0 + B_1 X_1 + B_2 X_2 + E$

Here we use B_0 to denote the intercept term, B_1 and B_2 to denote the coefficients for the two independent variables and E the sampling error. Given that the model has some theoretical justification, the statistician attempts to estimate the B coefficients. The statistician also explores the degree to which the regression model is a good model that fits the data [57 , 58].

When several independent variables are involved, the arithmetic computations can get very lengthy, and so it is not usually practical to undertake multiple regression without the aid of a computer. Several statistical packages such as SAS, BMD, and SPSS provide comprehensive programs that perform the calculations for multiple regression.

Age

Equation for Calculation of Age

The D/L ratio was expressed by the routine method [26] as $\ln[(1 + D/L)/(1 - D/L)]$. Estimated age was calculated using a regression line obtained by the least squares method using the D/L ratio as the x-axis and true age as the y-axis.

The rate equation was obtained using true age (t) as the x-axis and D/L ratio as the y-axis in a manner similar to that for the age calculation equation [52].

$\ln[(1 + D/L)/(1 - D/L)] = 2kt + \ln[(1 + D/L)/(1 - D/L)] = 0$

Here, k indicated the recemization reaction rate constant.

The standard errors (SE) are based on the difference between actual age and calculated (racemization) age.

Age determination for trace elements analysis:

$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + \ldots\ldots\ldots\ldots + B_{60}X_{60} + E$

Y = Constant + B_1*LnZn + B_2*LnPb + B_3*LnMn + B_4Ln*Sr + B_5Ln*Mg + B_6LnCu + B_7Ln*Fe.

Calculated age = EXP [y].

Discriminate analysis is a statistical technique in which linear combinations of variables are used to distinguish between two or more categories of cases. The variables "discriminate" between groups of cases and predict into which category or group a case falls, based upon the values of these variables.

The first task of discriminate analysis is to find the linear combination of variables that best discriminates between, or separates, groups. Frequently, discriminate analysis is used to classify a sample in which actual group membership is unknown. This can be done in two ways. We can combine two samples, one in which group membership is known and one in which group membership is unknown, into one sample. The cases for which group membership is known are used to compute the discriminate functions. Then discriminate functions are used to classify all cases or only those cases for which group membership is unknown. Alternatively, we can use discriminates to produce the matrix materials required for classification based upon one sample and then use these materials to classify the second sample.

To operate discriminates, we must specify a grouping variable, which identifies the group into which each case falls, and a set of discriminating variables. Since discrimination is capable of performing multiple analysis in which different sets of discriminating variables are used or in which different criteria are used for the entry or removal of variables, two separate subcommands are used to define the variables used in that analysis.

Gender

Gender was determined by Fisher's discrimination score of the analysis for sex determination. The following equation for each group was used:

$Y=A_1+C_{11}V_1+C_{21}V_2+\ldots+C_{61}V+.$ For Males

$Y=A_2+C_{12}V_1+C_{22}V_2+\ldots+C_{62}V+.$ For Females

Where Y= total score, A= the constant, C_{11}-C_{61} are the Fisher's discrimination scores for males, C_{12}-C_{62} are Fisher's discrimination scores for females and V_1-V_6 are the explanatory variables. A case is classified into the group that predicts the highest total classification score and computes the linear combination for each case. If males have a higher classification score that females then it will be males, and if females have a higher classification score that males then it will be females [57].

Chapter 3

MATERIALS AND METHODS

3.1 Introduction

Kuwait is a developing country and there is a high incidence of dental caries The mean DMFT for the whole adult population is 11.7. 46% have intensive gingivitis and 18 % have advanced periodontal involvement and often cervical caries [59 , 60]. Among persons aged over 30 years, advanced periodontal involvement was found in 35-57% of the population. In this experiment it was necessary to use non-carious teeth because the bacteria in caries may affect racemization (55) and the affect of caries on tooth trace elements is unknown. These factors factors restricted upon the number of teeth that could be collected.

Kuwait is an Islamic country and it is illegal to take teeth from bodies at postmortem or to dig in a cemetery for any experimental study.

The Kuwait Institute for Scientific Research helped locate soil similar to that of a cemetery, having similar conditions of, pH, humidity and temperature for trace element analysis. The temperature of the soil was recorded automatically and regularly (every hour) using an Omega thermocouple for 10 months at depths of 0.5 meters and 2.0 meters

The teeth were collected from the Department of Oral Surgery, Kuwait Dental Center. All teeth were healthy and free from restorations and dental caries. The teeth studied were extracted because of periodontal disease or for orthodontic reasons. Wisdom teeth (used for trace element analysis only) were extracted as partially impacted teeth in the oral surgical department. All the teeth used were extracted from living subjects of different ages, sex, medical conditions, occupation and nationality and all this personal data was recorded. The dates of extraction of the teeth were established and recorded. In order to remove residual soft tissue attached to the freshly extracted tooth the whole tooth was immersed in sodium hypochlorite NaOCl

(Clorox) solution [54, 56] at room temperature 24^0C for one hour after which the solution was replaced with a fresh solution of 10% (v/v) NaOCL and the tooth left in the solution for a further 24 hours. The soaking was carried out in a Class 2 microbiological chamber. The tooth was then repeatedly rinsed in deionised water (10 times) and then left to air-dry at room temperature. All teeth were then stored dry at -70^0C .

The teeth that were buried were buried in the desert at depths of 0.5 and 2.0 meters that approximated to the depths associated with a shallow, possibly criminal burial, and a legal Islamic burial. The teeth were buried for a period of 10 months in the soil similar to that of a cemetery. (Fig. 3 A) After making a hole in the crown, the teeth were tied to a heavy nylon fishing line and each fishing line was given a number for identification .

The Kuwait Institute for Scientific Research helped locate soil similar to that of a cemetery. The temperature of the soil was recorded regularly (every hour) using a thermocouple (Fig. 3 B). Soil samples were taken from 0.5m and 2.0m depth for analysis for mineral and trace elements.

Prior to determination of the racemization of aspartic acid and the level of trace elements in the dentine, both the fresh teeth which had not been buried and the teeth which had been buried for ten months teeth were washed with distilled water and then the crown and the apical third of the root were removed . The cement was removed with a dental drill and any residual pulp tissue carefully removed using endodontic reamers.

Figure 3 A digging in the soil similar to the cemetery.

Figure 3 B. The Thermocouple for recording the temperature regularly every hour.

3.2 The D- and L- aspartic acid analysis

3.2.1. Selection of not buried teeth for D- and L- aspartic acid analysis

Table 3.A. A total of 96 upper first premolar teeth were collected for amino acid analysis, 53 of these teeth (30 males and 23 females) were of ages and gender known to the writer and were used to establish a base-line for the determination of age and gender. (Control). A further 43 upper first premolar teeth (29 males and 14 females) of recorded age and gender, but which were unknown to the writer were used to test the methodology (Test).

Table 3.A The distribution of 96 upper first premolar teeth collected for amino acid analysis

No of teeth	Males	Females	Total
Control	30	23	53
Test	29	14	43
Total	59	37	96

3.2.2. Selection of buried teeth for D-and L- aspartic acid analysis

Table 3.B. A total of 45 buried first upper premolar teeth were use for D- and L aspartic acid analysis for age and gender determination.

14 of these teeth whose age and gender were known to the writer were buried in soil at a depth of 0.5 M ; (8 males and 6 females) and similarly 17 teeth (10 male and 7 female) were buried at 2M. This provided a total control group of 31 teeth. Six first upper premolar teeth of recorded age and gender (but unknown to the writer at the time of the experiment) were buried in the soil at a depth of 0.5M and similarly 8 teeth were buried at 2M thus providing a small test group of 14 teeth.

Table 3.B The distribution of 45 first premolar teeth

Buried 0.5m.				Buried 2.0m			
	Male	Female	Total		Male	Female	Total
Control	8	6	14	Control	10	7	17
Test	3	3	6	Test	6	2	8
Total	11	9	20	Total	16	9	25

3.2.3. Selection of not buried teeth used for D-and L-aspartic acid and trace element analysis.

The 53 first upper premolar teeth (30 males and 23 females) were bisected vertically and one half used for amino acid analysis and the other half for trace element analysis. (controls)

Table 3.C. The distribution of 53 first upper premolar teeth

Total	Male	Female
53	30	23

3.2.4. Chemicals for D- and L- aspartic acid procedures.

All the chemicals used for the assay of D- and L- aspartic acid were produced by SIGMA unless otherwise stated. All solvents were HPLC grade.

Dowex 50x8 (Na) size 0.3-0.85 mm (18-52 mesh) standard grades No 55036 BDH chemicals Ltd. Pool England. Sodium Borate (Borax) Decahydrate ACS Reagent, Sigma Lot No 116H0012) Assay 99.9 % pH 9.16 at 25C FW 381.4. Sodium Acetate Anhydrous, Sigma (S-8750, Lot No 76H0075) minimum 99.0% FW 82.02. Sodium Chloride, BDH, Lot No 12261/89732. Lauryl Sulfate (Sodium dodecyl Sulfate) Sodium Salts 99.0%, Sigma, Lot No 57H1242. 6-Amino-n-hexanoic acid, Sigma, Minimum 99.0% Lot No 86H07821. Phenylmethyl sulfonyl Flurried, Sigma, Lot No 18H5018. L-aspartic acid standard. > 99.0% (TLC) sigma, A-8949 Lot No 77H1398. D-Aspartic acid standard, minimum 99.9% (TLC), Sigma, A-8881 Lot No 74H5047.

O-phthaldialdehyde, Approx., 99.0% FW 134.1, Sigma, P-0657, Ig, Lot No 116H5030. N-Acetyl-L-Cysteine, >99.0% (TLC), Sigma, A-8199 Lot No 87H03951. Methanol Isocratic HPLC grade, Scharlau (Me 308, Batch No 27071). Ammonium Hydroxide ACS reagent FW 35.05, Sigma, A-6899 Lot No 116H3418. Ether. Ethylene. 6-NHCL.

3.2.5 Apparatus for D- and L- aspartic acid analysis

The HPLC system consisted of a model 600s controller. Model 626 pump. Model 717 plus autosampler. Model 474 scanning fluorescence detector. Reversed phase HPLC column model Kromasil C8, 5um and 4.6x250 mm-analytical column pat No p51847275 made in USA. Research analytical oven (Harvard Ltd. Greenbridge Lane, Greenfield, Oldham, Lances, OL37EN, England) was used for hydrolysis. Vacuum system model (Hetovac Vacuum Rotator VR-1) attached via a cold system model (Hetotrap Cooling Trap Ct 60/90/110) and a high vacuum pump (Heto Lab Equipment cooling trap CT 110 ID: 872013) were used for drying purpose (see Figure 3.C)

Figure 3.C The different parts of HPLC system.

1. Waters 474 scanning fluorescence detector Millipore.
2. Waters 600 S controller Millipore.
3. Waters 996 photodiode array detector Millipore.
4. Water 626 pump
5. Waters 717 autosampler
6. Aspire Acer computer system with Millipore software HPLC analyzer.

3.2.6 Production of shards for D- and L- aspartic acid analysis

Each dentine sample for analysis was frozen in liquid nitrogen for 4 to 6 minutes, then immediately wrapped in a fine nylon bag and crushed into a powder in a pestle and mortar. The resulting powder was weighed [62].

3.2.7 Tooth washing for D- and L-aspartic acid analysis

The resulting shards were prepared by a method essentially the same as that described by Takagi & Veis 1984 [62]. 40-80 mg of each resulting dentine powder was washed in 15% NaCl at 4^0C overnight on a rocking table. The resulting shards were washed in 15% NaCl containing neutral protease inhibitors (Sigma) at 4^0C overnight with stirring. The following day, the washed samples were centrifuged at 4^0C for 5 min at 13.000 rpm in a microcentrifuge. The supernatant was removed and discarded. The pellet was rinsed with ethanol ether (3:1 by volume) for 7 min, centrifuged as before and the supernatant removed and discarded. The pellet was then soaked in 2% of sodium dodecyl sulfate for 60 minute at room temperature. The pellet was then dried in a vacuum pump.

3.2.8 Hydrolysis for D- and L- aspartic acid analysis

The dry residue recovered from the washing step was resuspended in 1 ml of 0.6 N HCL at 110'C for 9 hours as described by Carolan et al 1997 [56] in preference to 108'C for 20 hours as described by Shimoyama and Harada in 1984 [63].

The samples were transferred to a long glass tube using Pasteur pipettes. The samples were hydrolyzed in a glass test tube with Pete seals in the lid at 100^0C for 6 hrs in the oven. They were then resuspended in 1 ml of distilled and deionized water and desalted on an action exchange column.

Longer hydrolysis times will induce racemization [31], and this must be accounted for when comparing rates across studies. The activation energy of hydrolysis is higher than that of racemization, so shorter hydrolysis times at higher temperatures will induce less racemization for the same amount of peptide bond hydrolysis [64].

3.2.9 Chromatographic analysis method:

To separate amino acid enantiomers, high performance liquid chromotography (HPLC) has been used, and in many respects is better than gas chromotography (GC). The effectiveness of GC and HPLC in separating aspartic acid have been compared and HPLC was considered to be better [54] HPLC with D-leucine N-carboxyandride as an ultraviolet derivatized reagent to measure the d-aspartic acid in dental collagen has been used for age estimation [54] and the results for fresh teeth were encouraging. The procedures for separating aspartic acid are laborious and time consuming [64]. We used a simple and sensitive method for D/L- analysis with a reverse phase HPLC column and fluorescence detection. We have applied this method to determine the D/L ratio of aspartic acid in dentine and used it to estimate the age at death of the samples.

3.2.10 Desalting for D- and L- aspartic acid analysis

The samples were desalted on a microcolumn using Dowex 50-x8 resin (particle size 0.3-0.08 mm, 18-52-mesh size BDH) to remove particular matter and inorganic salts. The resin was soaked in 6MHcl for 6 hr and then rinsed again with distilled water to completely remove all the acid. Pasteur pipettes were used as mini columns with prextracted plugs of glass wool at the bottom. They were filled with resin to about two thirds full, taking care not to allow air into the column. The resin was washed with 3 volumes of distilled water to remove the salts and then the amino acid was eluted with 3 volumes of 2M ammonium hydroxide. The elute was then divided into four aliquots (1.5 ml each). Each of these aliquots were dried, weighed and frozen at -70^0C.

3.2.11 Derivatisation of the Sample for HPLC

One of the 4 aliquots was reconstituted with deionised distilled water for derivatisation and another 3 were frozen dry at -70^0C for future analysis. Derivatisation was accomplished by mixing 10 μ l of prepared sample solution with 20 μ l of OPA-NAC reagent in a small test tube (OPA-NAC regent was prepared as follow: 8.0 mg of O-pthaldehyde was dissolved in 600 μ l of methanol, 500 μ l of 0.4 mol/ L Na borate (pH 9.4) was added, 800 μ l of distilled water was added, 120 μ l of

1.0 mol/L N-Acetycysteine was added). The OPA-NAC regent was stored at 4^0C. After 2.5 min, 200 μ l of 50 mMol Na acetate was added, then 10 μ l of the solution was taken for direct injection into the HPLC system.

3.2.12 HPLC Operation Condition

The HPLC system (Waters, Millipore) consisted of pump and a model 474 scanning fluorescence detector. The diastereomeric dipeptides were separated on a reversed phase kromasil C8 (25cm *4.6mm) column placed after a guard column.

Isocratic elution with 90% solvent A and 10% solvent B was maintained for 5 min. Subsequently, solvent B was increased linearly to 100% over a period of 5 min. Solvent A was 50 mm sodium acetate, pH 5.9 and solvent B was 80% (v/v) methanol and 20% solvent A. Throughout the run, flow rate was maintained at 1.0ml/min. The excitation wavelength was set at 340nm and the emission wavelength was at 420nm. D- and L- aspartic acid were eluted at about 8 and 10 min respectively and the remaining compounds were collected between 13 and 20 min. Standard D- and L-aspartic acid were run individually and also in a mixture along with a set of samples. The ratio of D- and L-aspartic acid was calculated from the areas under the eluted peaks.

The racemization of amino acids follows a first order reversible rate law equation, which is as follow: $\text{Ln}\,(1+D/L) / (1-D/L) = 2Kt+A$

Ln is the natural logarithm, D/L is the ratio of the area of eluted peaks of D and L aspartic acids, 2K is the constant (slope) of racemization and can be calculated from a linear regression of $\text{Ln}\,(1+D/L) / (1-D/L)$ Vs age of the individual, t is the true age (year) of the individual, A is the intercept of the linear regression line with Y axis [13 14,54,61 ,65].

3.3 Trace element analysis

3.3.1. Selection of not buried teeth for trace element analysis

Table 3.3.1. A total of 453 Kuwaiti teeth were used for trace element analysis.

300 (control) different teeth (140 males and 160 females) were divided into 7 groups depending on their age. The age distribution was as follows: 10-14 years, 15-19 years, 20-24 years, 25-29 years, 30-34 years, 35-39 years, 40-64 years. 100 different

teeth of known age and gender (but unknown at the time of the experiment to the writer) were used for age and gender estimation from trace element analysis (Test).In addition a further 53 upper first premolar teeth were bisected vertically and one half used for amino acid (racemization) studies and the other half for trace element analysis.

Table 3.3.1 The distribution of 400 different teeth

No of teeth	Males	Females	Total
Control	140	160	300
Test	45	55	100
Total	185	215	400

3.3.2. Selection of buried teeth for trace element analysis

Table 3.3.2 A total of 69 Kuwaiti third molar tooth were used for trace element analysis for age and gender determination. 19 of these teeth whose age and gender were known to the writer were buried in soil to a depth of 0.5M (12 males and 7 females) and similarly 16 teeth (10 males and 6 females) were buried at 2M. This provided a control group of 35 teeth. 18 teeth of recorded age and gender (but unknown to the writer) were buried at a depth of 0.5M and similarly 16 teeth were buried at 2M thus providing a test group of 34.

Table 3.3.2. The distribution of 69 first premolar teeth.

Buried 0.5M		Buried 2.0M		Total
Control	19	Control	16	35
Test	18	Test	16	34
Total	37	Total	32	69

3.3.3 Chemicals for trace element analysis

Concentrated nitric acid (HNO3) M=63.01 g/mol (Riedel de Haea Germany Un No 2031). Palladium Atomic Absorption standard solution 1010 ug/ml in 5% HCL (Sigma P-4400). Selenium, Manganese, Lead and Strontium Atomic Absorption standard solution 1000 PPM each (Solution Plus Inc USA). Zinc, Copper, Mg and Iron Atomic Absorption standard solution 1000 PPM (Fluka). Concentration Triton X-100 for washing the instrument (BDH Chemicals, Pool UK). Argon used as purge gas in Graphite Furnace AA. Air-acetylene used as purge gas in flame burner. Double distilled water (Milli-Qreagent water system, Millipore, Milford MA). 30% HNO3 and 30% HCL for cleaning of all the glassware.

3.3.4 Apparatus for trace element analysis

Atomic absorption spectrophotometer flame (Varian Spectra AA 300/400 Australia) for detecting concentration of Zn, Fe and Mg . Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) with Zeeman correction (Varian model, Spectra AA 800 GTA Australia) for detecting Mn, Pb, Cu and Sr (figures 3.D - F). 10 ml and 50 ml polystyrene tubes equipped with loosely fitting Teflon caps. Polystyrene sample cups. Automated pipettes equipped with disposable polypropylene pipette tips.

3.3.5 Drying and hydrolyization of dentine for trace element analysis

Fresh dentine samples (0.1-0.5g) were dried at about 105^0C in an oven for a maximum of 24 hours [41 , 42]. In order to determine whether they were completely dried, each sample was weighed at four different intervals and the final dried weight of each sample was recorded.

Hydrolyzation of dentine was performed in special metal-free vacationer glass tubes (B & D Royal Blue). Hydrolyzation was carried out in concentrated nitric acid (HNO_3). The samples were incubated overnight in an oven at 60^0C until the dentine was completely digested. The following day, the clear supernatants were transferred into special metal free graduated plastic tubes and diluted up to 25ml with distilled

and deionized water (ddw) [42]. The diluted samples were preserved at -4^0C for further analysis.

3.3.6 Dentine trace element analysis

Two atomic absorption spectrophotometers were used (Varian Spectra AA model 300/400 with a flame atomization and Varian Spectra AA model 800 with an atomizing graphite furnace and Zeeman correction). Prior to determination of the level of Zn, Cu, Mg, Fe Mn, Pb and Sr, the instruments were standardized for each element according to the operating parameters described in the manufacturer's operating manual. The standards prepared in 4% nitric acid were run in the range as shown in Table 3.3.6.

Table 3.3.6 The range of calibration for different trace elements.

Element	Range of Calibration (PPM)
Zn	0.1-0.4
Fe	0.1-0.5
Mg	0.2-1.0
Mn	1.0-5.0
Sr	3.0-9.0
Cu	15.0-45.0
Pb	10.0-40.0

Levels of Zn, Fe and Mg were estimated using an air acetylene burner with hollow cathode lamps whereas the levels of Mn, Pb and Sr, the concentrations of which are very low in the dentine, were determined by a very sensitive graphite atomizer with Zeeman correction (Varian model AA 800 atomic absorption spectrophotometer). A modifier that maintains the stability of the analyzing elements at a higher temperature (20mg citric acid in 2ml palladium 500ppm) was used for the analysis of Pb concentration in the dentine. The modifier improves the determination of the level of volatile elements in difficult sample matrices by reducing the background absorbency and chemical interference. For determination of Mn, Pb, Cu

and Sr, the standard addition method was used. Argon gas and hollow cathode lamps were used for atomization and absorption of ionic elements in the furnace graphite atomizer. All samples were diluted with distilled water and contained the same acid concentration as the standards. The dilutants were analyzed for any possible trace element contamination.

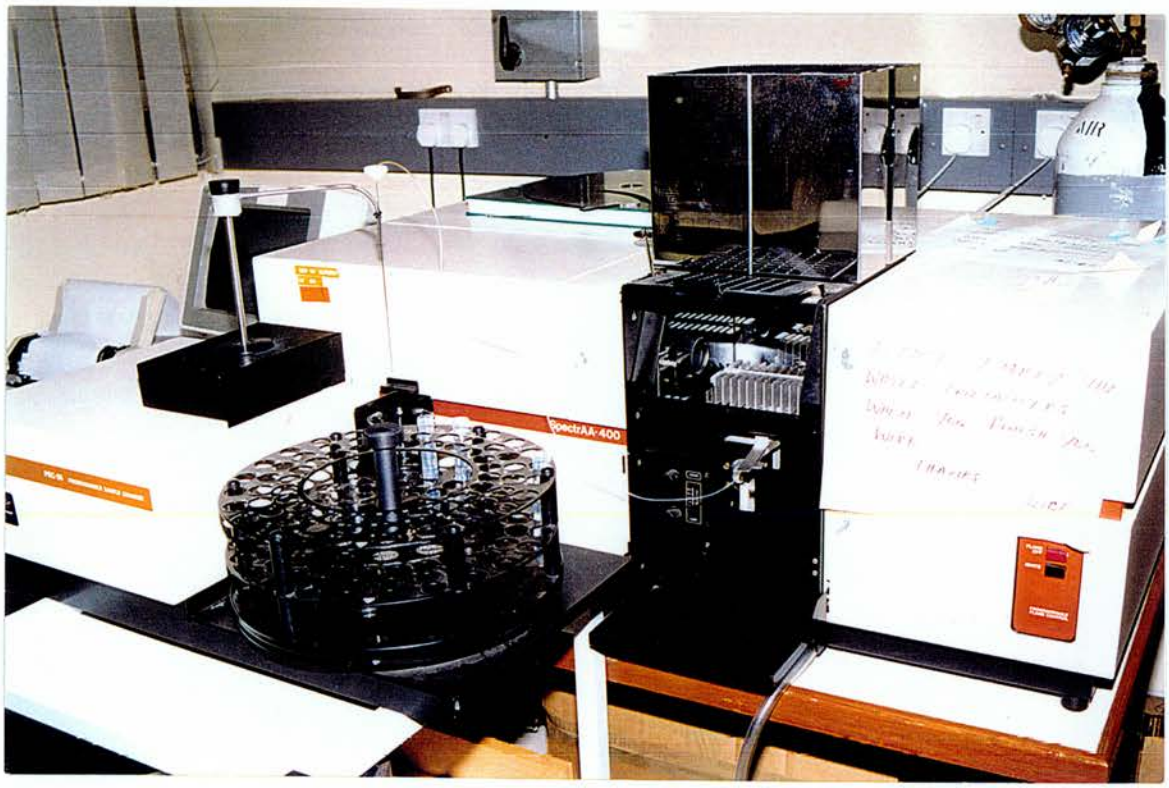

Figure 3.D. The flame Atomic Absorption Spectrophotometer (Spectra AA 400) for detecting concentration of Zn, Fe and Mg.

Figure 3.E. The Atomic Absorption Spectrophotometer (Spectra AA 800)

Figure 3.F The Spectra AA GTA 100 sampler and processor.

3.4. The Statistical Analysis

The package SPSS/PC professional statistics version 8.0 for Windows was used for data analysis. A linear regression model was used for age determination. A logarthim transformation was carried out for trace elememt Gaussian (normal) calculation. Discriminate analysis was used for gender determination .

3.4.1 Age determination for D/L aspartic acid.

Equation for Calculation of Age

The D/L ratio was expressed by the routine method [29] as $1n[(1 + D/L)/(1 - D/L)]$. Estimated age was calculated using a regression line obtained by the least squares method using the D/L ratio as the x-axis and true age as the y-axis.

The rate equation was obtained using true age (t) as the x-axis and D/L ratio as the y-axis in a manner similar to the for the age calculation equation:

$1n [(1 + D/L)/(1 - D/L)] = 2kt + 1n[(1 + D/L)/((1 - D/L)] = o$

Here, k indicates the racemization reaction rate constant.

The rate equation was obtained using estimated age (t) for unknown age .

3.4.2 Age determination for trace elements analysis

The purpose was to study the effect of changes in one element (x_1) on another (y). When it was recognized that y is affected by several other elements (x_2, x_3, etc.), it was necessary to study the effect of simultaneous changes in x_1, x_2, etc. on y. The appropriate technique when dealing with continuous variables is multiple linear regression analysis.

$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + \ldots\ldots\ldots\ldots + B_{60}X_{60} + E$

Y = Constant + B1*LnZn + B2*LnPb + B3*LnMn + B4*LnSr + B5*LnMg + B6*LnCu + B7*LnFe.

Calculated age = EXP[y].

3.4.3 Gender was determined by Fisher's discrimination score of the analysis for sex determination. The following equation for each group was used:

$Y=A_1+C_{11}V1+C_{21}V_2+\ldots+C_{61}V_6+$. For Males

$Y=A_2+C_{12}V_1+C_{22}V_2+\ldots+C_{62}V_7+$. For Females

Where Y= total score, A= the constant, C11-C61 are the Fisher's discrimination scores for males, C_{12}-C_{62} are Fisher's discrimination scores for females and V1-V6 are the explanatory variables. A case is classified into the group that predicts the highest total classification score and computes the linear combination for each case. If males have a higher classification score that females then it will be males; and if females have a higher classification score that males then it will be females.

3.5 Soil analysis

In this study teeth were buried in soil similar to the cemetery soil at depths of 0.5M and 2.0 Ms.

Samples of soil from these depths were examined extensively by The Kuwait Institute for Scientific Research. 76 soil sample were dug according to a 3 x 3 Km grid pattern to investigate the soil properties.

The pH was examined for 76 soil samples at both depths. The humidity was examined for 76 soil sample at both depths by measuring the difference in weight of soil samples before and after heating . The temperature of the soil was recorded regularly (every hour) 24 hour a day for 10 months by using an Omega automatic thermocouple.

The concentration of trace elements was examined for the 76 soil samples at both depths. This was done to examine their effect on the buried teeth. Quantitative determination of the trace elements in the soil was carried out as follows: -

1. **Homogenization:** - It was necessary that the soil sample was homogenized for analysis. Homogenization can be achieved by quartering, rolling and shaking a large portion of the sample so that a small portion of the sample can be taken from it for analysis.
2. **Separation Method:** - In this method, a 200-mesh sieve separated fine particles from the soil.

3. **Drying:** - In this stage the sample was dried in an oven at temperatures ranging from 75 -105^{0}C.
4. **Sample treatment:** - For total metal determination, 1-2 grams. of the sample was taken in a platinum crucible wetted with water and digested with 0.5ml of 72% perchloric acid and 5ml of 48% hydrofluoric acid on a sand bath at 200-225^{0}C. Care was taken to ensure that the crucible was partially covered with a platinum lid so that the sample did not dry out. The residue was taken out by boiling the sample with 5 ml of 60% hydrochloric acid and 10-15 ml of water. The contents of the crucible were then transferred to a 100ml volumetric flask by washing of the crucible and lid.
5. **Standard preparation:** - Aqueous standard solutions were prepared (1000ug/ml).
6. **Chemical modifier:** - 1-2% ammonium dihydrogen phosphate or 3% phosphoric acid was used as a chemical modifier for most elements in the soil.
7. **Calibration procedure:** - Normal calibration against three standards of the elements of interest in the same acid as the sample was calibrated and the atomic absorption spectrometer (Furnace AAS) or (Flame AAS) was used for most elements.

Temperature was recorded at depths of 0.5M and 2.0M by using a thermocouple every hour from January to October 1998 (a period of 10 months).

Chapter 4

RESULTS

4.1 Determination of age by D- and L- aspartic acid racemization from teeth not buried in the desert

Tables 4.1.1 a – c . There were a total of 96 Kuwaiti upper first premolar teeth taken for amino acid analysis and of these 53 teeth were not buried in the desert. The age ranged from 10.0 to 45.0 years. For 30 males, the age ranged from 10.0 to 27.0 years, and for 23 females the age ranged from 10.0 to 45.0 years. The teeth were grouped into 5 groups depending on their age. The age distribution was as follows: 1: 10-15 years, 2: 16-21 years, 3: 22-27 years, 4: 28-33 years, 5: 34-39 years and 6: 40-45 years.

43 upper first premolar teeth (29 males and 14 females) of recorded age and gender but which were unknown to the writer were used to test the methodology.

Table 4.1.1.a. The distribution of age groups for 30 males

Age Groups	No	Minimum Age	Maximum Age
1. 10-15	22	10.00	15.08
2. 16-21	7	16.02	18.05
3. 22-27	1	0	0
4. 28-33	0	0	0
5. 34-39	0	0	0

Table 4.1.1.b. The distribution of age groups for 23 females

Age Groups	No	Minimum Age	Maximum Age
1. 10-15	11	10.00	15.06
2. 16-21	3	17.00	20.08
3. 22-27	4	23.00	27.00
4. 28-33	1	0	0
5. 34-39	4	34.00	39.50

Table 4.1.1.c. The distribution of age groups for 53 males and females.

Age Groups	No	Minimum Age	Maximum Age
1. 10-15	33	10.00	15.08
2. 16-21	10	16.02	20.08
3. 22-27	5	23.00	27.00
4. 28-33	0	0	0
5. 34-39	4	34.00	45.00

Table 4.1.2. Racemization of aspartic acid in 53 upper first premolar teeth not buried in the soil.

No	Sex	Age	D/L	No	Sex	Age	D/L
1	M	16.02	0.016	28	M	18.04	0.021
2	M	14.00	0.013	29	M	14.00	0.013
3	F	15.02	0.017	30	M	27.00	0.033
4	M	12.04	0.011	31	M	15.00	0.016
5	M	15.02	0.016	32	M	12.09	0.011
6	F	14.02	0.014	33	F	19.06	0.027
7	M	11.01	0.009	34	M	14.02	0.014
8	F	17.11	0.020	35	M	17.00	0.019
9	M	15.08	0.017	36	F	14.01	0.014
10	F	15.00	0.015	37	F	12.00	0.011
11	M	10.00	0.005	38	M	12.08	0.011
12	F	10.04	0.006	39	M	17.07	0.019
13	M	11.00	0.009	40	F	14.00	0.014
14	M	11.02	0.010	41	M	15.00	0.017
15	M	11.02	0.010	42	F	23.00	0.024
16	F	26.00	0.032	43	F	23.00	0.026
17	M	13.01	0.012	44	M	12.00	0.011
18	F	14.08	0.015	45	F	13.00	0.011
19	F	27.00	0.035	46	M	18.05	0.022
20	M	10.09	0.008	47	M	16.02	0.018
21	F	15.06	0.017	48	F	45.00	0.059
22	M	11.11	0.011	49	F	38.00	0.048
23	F	34.00	0.004	50	F	10.00	0.007
No	Sex	Age	D/L	No	Sex	Age	D/L
24	M	13.04	0.013	51	M	14.00	0.015
25	M	10.08	0.008	52	F	32.00	0.037
26	F	20.08	0.030	53	F	41.00	0.051
27	M	16.03	0.018				

Table 4.1.3.a-d and figure 4.1.3–a-c shows the Quantitative analysis ratio based on a method described by Ohtani and Yamamoto 1987 [52] for the 53 upper first premolar teeth. The linear regression and correlation coefficients (R square) were calculated for the relationship between the extent of aspartic racemization and actual age (t).

The relation between actual age and D/L ratio for 53 upper first premolar teeth not buried in the soil.

$[\ln(1+D/L)/(1-D/L) = 0.002903\ t + (-0.01228)]$ 1

$t=[\ln(1+D/L)/(1-D/L)+0.01228)/(0.002903)]$ R square = 97.8 %.

To calculate the SE (standard error of estimation) was investigated by descriptive analysis between the actual age – racemization age .

SE = 1.2 years.

For 30 male

$t=[\ln(1+D/L)/(1-D/L) + 0.01552)/(0.003120)]$ ----------(2)

R square = 97.1 % SE = 0.59 years.

For 23 female

$t= [\ln(1+D/L)/(1-D/L) + 0.01096)/(0.002853)]$ ----------(3)

R square = 97.3 % SE = 1.7 years.

Table 4.1.3.a An age determination for 53 upper first premolar teeth not buried

NO	Actual age	Ln(1+D/L) / (1-D/L)	Error years	NO	Actual age	Ln(1+D/L) / (1-D/L)	Error years
1	16.02	15.79	0.23	28	18.04	17.42	0.62
2	14.00	13.17	0.83	29	14.00	13.47	0.53
3	15.02	15.38	-0.36	30	27.00	26.47	0.53
4	12.04	12.19	-0.15	31	15.00	14.68	0.32
5	15.02	14.94	0.08	32	12.09	12.37	-0.28
6	14.02	14.02	0.00	33	19.06	19.31	-0.25
7	11.01	11.28	-0.27	34	14.02	13.85	0.17
8	17.11	17.10	0.01	35	17.00	16.96	0.04
9	15.08	15.56	-0.48	36	14.01	13.64	0.37
10	15.00	14.56	0.44	37	12.00	12.03	-0.03
11	10.00	8.80	1.20	38	12.08	12.12	-0.13
12	10.04	9.44	0.60	39	17.07	16.72	0.35
13	11.00	11.11	-0.11	40	14.00	13.74	0.26
14	11.02	11.33	-0.31	41	15.00	15.49	-0.49
15	11.02	11.47	-0.45	42	23.00	22.53	0.47
16	26.00	26.18	-0.18	43	23.00	23.42	-0.42
17	13.01	12.73	0.28	44	12.00	12.05	-0.05
18	14.08	14.51	-0.43	45	13.00	12.35	0.65
19	27.00	27.38	-0.38	46	18.05	17.67	0.38
20	10.09	10.70	-0.61	47	16.02	16.38	-0.36
21	15.06	15.62	-0.56	48	45.00	46.16	-1.16
22	11.11	11.98	-0.87	49	38.00	37.76	0.24
23	34.00	35.02	-1.02	50	10.00	9.77	0.23
24	13.04	13.03	0.01	51	14.00	14.28	-0.28
25	10.08	10.26	-0.18	52	32.00	31.50	0.50
26	20.08	20.49	-0.41	53	41.00	40.05	0.95
27	16.03	16.65	-0.62				

Table 4.1.3.b An age determination for 30 upper first premolar male teeth not buried

NO	Actual age	Ln(1+D/L) / (1-D/L)	Error years	NO	Actual age	Ln(1+D/L) / (1-D/L)	Error Years
1	16.02	15.79	0.23	16	16.03	16.65	-0.62
2	14.00	13.17	0.38	17	18.04	17.42	0.62
3	12.04	12.19	-0.15	18	14.00	13.47	0.53
4	15.02	14.94	0.08	19	27.00	26.47	0.53
5	11.01	11.28	-0.27	20	15.00	14.68	0.32
6	15.08	15.56	-0.48	21	12.09	12.37	-0.28
7	10.00	8.80	1.20	22	14.02	13.85	0.17
8	11.00	11.11	-0.11	23	17.00	16.96	0.04
9	11.02	11.33	-0.31	24	12.08	12.21	-0.13
10	11.02	11.47	-0.45	25	17.07	16.72	0.35
11	13.01	12.73	0.28	26	15.00	15.49	-0.49
12	10.09	10.70	-0.61	27	12.00	12.05	-0.05
13	11.11	11.98	-0.87	28	18.05	17.67	0.38
14	13.04	13.03	0.01	29	16.02	16.38	-0.36
15	10.08	10.26	-0.18	30	14.00	14.28	-0.28

Table 4.1.3.c An age determination for 23 upper first premolar female teeth not buried

NO	Actual age	Ln(1+D/L) / (1-D/L)	Error years	NO	Actual age	Ln(1+D/L) / (1-D/L)	Error years
1	15.02	15.38	-0.36	13	14.01	13.64	0.37
2	14.02	14.02	0.00	14	12.00	12.03	-0.03
3	17.11	17.10	0.10	15	14.00	13.74	0.26
4	15.00	14.56	0.44	16	23.00	22.53	0.47
5	10.04	9.44	0.60	17	23.00	23.42	-0.42
6	26.00	26.18	-0.18	18	13.00	12.35	0.65
7	14.08	14.51	-0.43	19	45.00	46.16	-1.16
8	27.00	27.38	-0.38	20	38.00	37.76	0.24
9	15.06	15.62	-0.56	21	10.00	9.77	0.23
10	34.00	35.02	-1.02	22	32.00	31.50	0.50
11	20.08	20.49	-0.41	23	41.00	40.05	0.95
12	19.06	19.31	-0.25				

Table 4.1.3.d . The correlation (R square) and the standard errors of age estimation.

	Mean of Known Age	Mean of ln (1+D/L)/(1-D/L) Age	R square %	SE Years (mean)
30 Male	14.03	14.03	97.1	±0.59
23 Female	21.36	21.37	97.3	±1.71
53 Total	17.21	17.42	97.8	±1.21

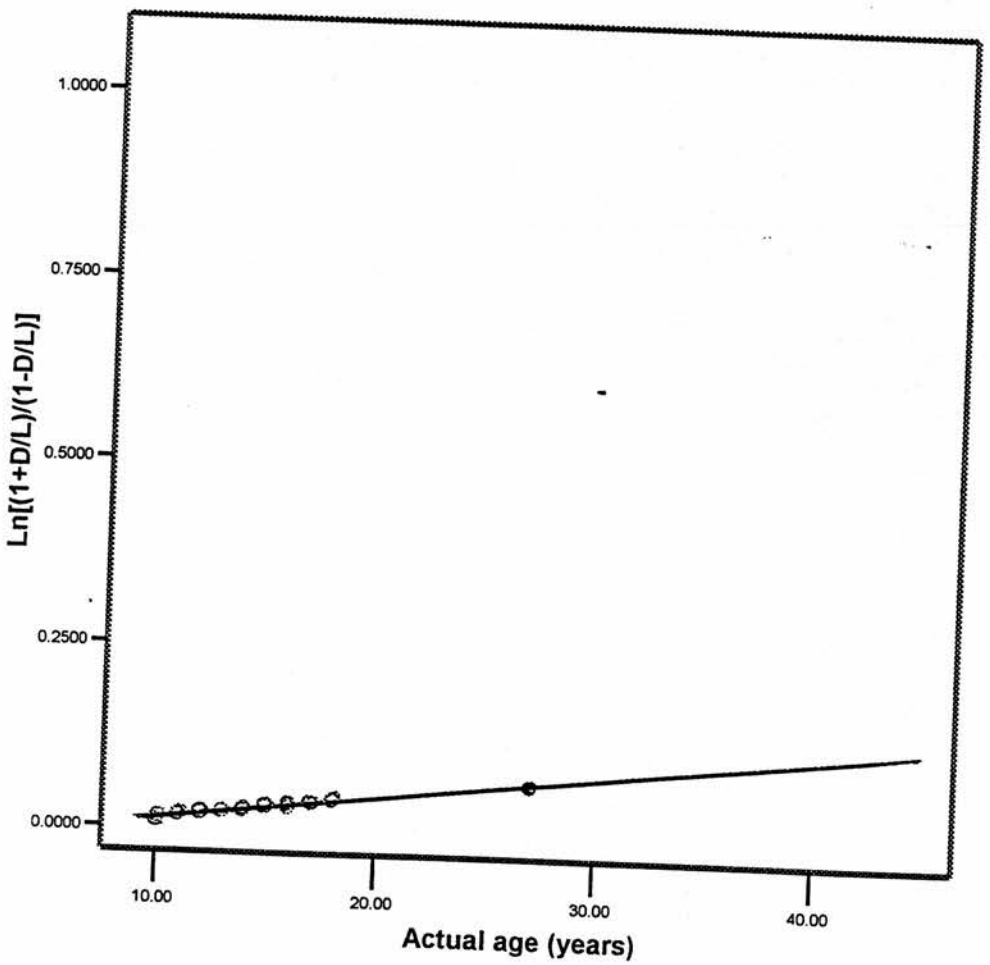

Figure 4.1.3.a The relationship between age and D/L ratio for 30 Male in Relation to Actual Age

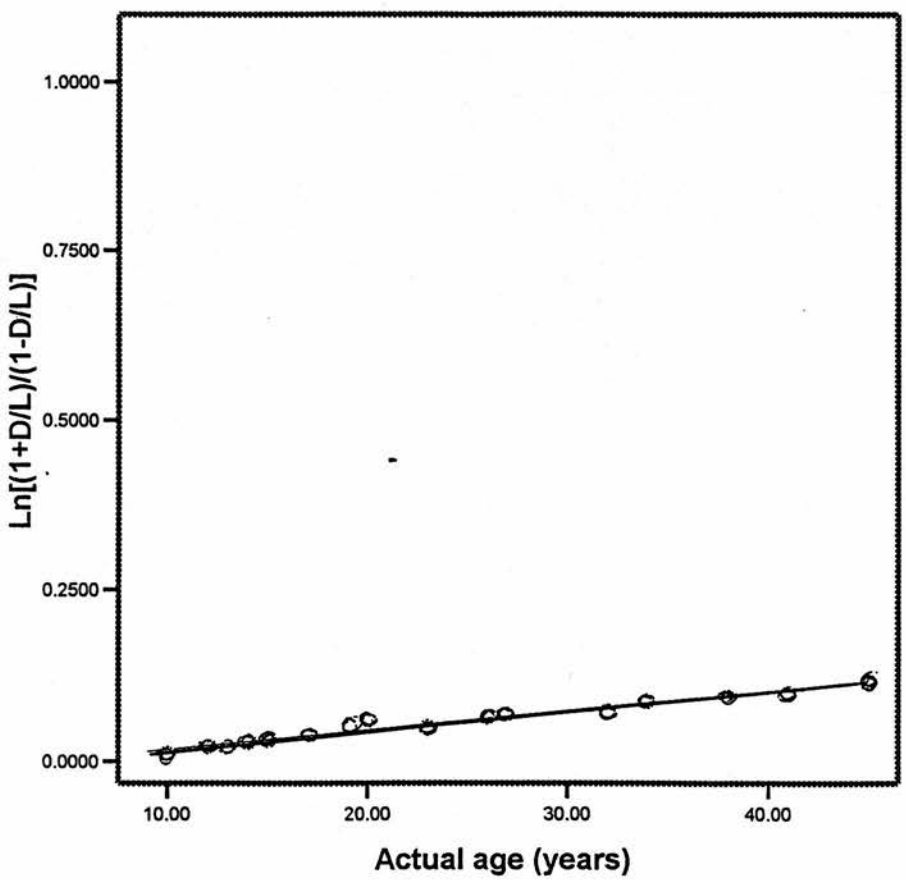

Figure 4.1.3.b The relationship between age and D/L ratio for 23 Females Relation to Actual Age

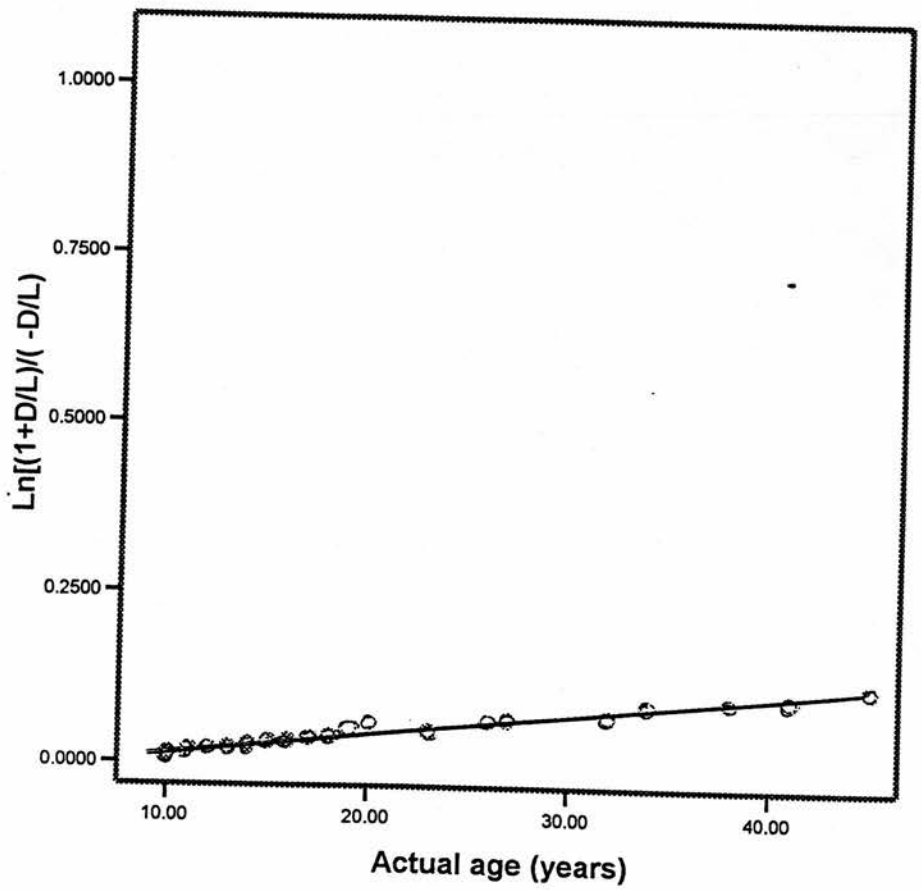

Figure 4.1.3.c The relationship between age and D/L ratio for Males and Females in Relation to Actual Age

Table 4.1.4. Shows the values obtained for the extent of aspartic acid for age groups in 53 upper first premolar teeth not buried in the soil.

Table 4.1.4. An age determination for age groups in 53 teeth not buried

Age Group Years	Mean of Known Age	**Mean of ln(1+D/L)/(1-D/L) Age**	R square %	SE
10 – 15	12.78	12.79	93.9 %	0.46
16 - 21	17.44	17.45	92.1 %	0.40
22 – 27	25.20	25.20	95.2 %	0.46
34 – 39	39.50	39.42	95.4 %	1.51

Application to Test teeth

Table 4.1.5. a-c , Table 4.1.6.a-c and Figure 4.1.6.a-c Show the application of age estimation for 43 upper first premolar teeth not buried in the soil by using the calculated formula derived from data in Table 4.1.2

Estimated age = [((1+D/L)/(1-D/L) + 0.01228)/(0.002903)]
The SE of control group was = ± 1.2 years
known age = estimated age + SE (± 1.2)

For males the application of age estimation for 29 upper first premolar teeth not buried in the soil by using the calculated formula .

Estimated age = [((1+D/L)/(1-D/L) + 0.01552)/(0.00312)]
The SE of control group was = ± 0.59 years
Known age = Estimated Age + SE (± 0.59)

For females the application of age estimation for 14 upper first premolar teeth not buried in the soil by using the calculated formula .

Estimated Age = [((1+D/L)/(1-D/L) + 0.01096)/(0.002853)].

The SE of control group was = ± 1.7 years

Known Age = Estimated Age + SE (± 1.7)

Table 4.1.5.a. The age estimation for 43 upper first premolar teeth not buried in the soil

No	Actual Age	Estimated age	No	Actual Age	Estimated age
1	16.02	15.79±1.2	23	10.08	10.26±1.2
2	14.00	13.17±1.2	24	25.00	25.65±1.2
3	15.02	16.13±1.2	25	17.00	16.65±1.2
4	12.04	12.19±1.2	26	18.04	17.42±1.2
5	15.00	14.94±1.2	27	14.00	13.47±1.2
6	11.01	11.28±1.2	28	25.90	26.47±1.2
7	18.00	17.10±1.2	29	16.00	15.73±1.2
8	15.08	15.56±1.2	30	12.09	12.37±1.2
9	14.10	14.56±1.2	31	24.00	23.73±1.2
10	9.00	8.80±1.2	32	14.02	13.85±1.2
11	10.04	9.44±1.2	33	17.00	16.96±1.2
12	11.00	11.11±1.2	34	14.01	13.64±1.2
13	11.02	11.33±1.2	35	12.00	12.03±1.2
14	11.02	11.47±1.2	36	12.09	12.21±1.2
15	13.01	12.73±1.2	37	17.07	16.72±1.2
16	14.08	14.52±1.2	38	14.00	13.74±1.2
17	27.00	27.38±1.2	39	12.00	12.05±1.2
18	10.09	10.70±1.2	40	13.00	12.35±1.2
19	15.05	15.62±1.2	41	18.05	17.61±1.2
20	11.11	11.98±1.2	42	17.00	16.38±1.2
21	34.00	35.02±1.2	43	14.02	14.02±1.2
22	13.04	13.03±1.2			

Table 4.1.5.b The age estimation for 29 upper first premolar male teeth not buried in the soil

No	Actual Age	Estimated age	No	Actual Age	Estimated age
1	16.02	15.1±0.59	16	13.04	13.0±0.59
2	14.00	13.2±0.59	17	25.00	25.14±0.59
3	12.04	12.1±0.59	18	17.00	16.8±0.59
4	15.00	15.2±0.59	19	18.04	18.3±0.59
5	11.01	11.0±0.59	20	14.00	13.5±0.59
6	15.08	16.0±0.59	21	25.90	26.1±0.59
7	9.00	8.16±0.59	22	16.00	14.9±0.59
8	11.00	10.8±0.59	23	14.02	14.0±0.59
9	11.02	11.1±0.59	24	17.00	17.04±0.59
10	11.02	11.2±0.59	25	12.09	12.1±0.59
11	13.01	12.5±0.59	26	17.07	16.9±0.59
12	10.09	10.3±0.59	27	12.00	11.9±0.59
13	15.05	15.92±0.59	28	18.05	18.8±0.59
14	11.11	11.9±0.59	29	17.00	16.2±0.59
15	34.00	33.5±0.59			

Table 4.1.5.c. The age estimation for 14 upper first premolar female teeth not buried in the soil

No	Actual Age	Estimated age	No	Actual Age	Estimated age
1	15.02	15.62±1.7	8	12.09	12.3±1.7
2	18.00	17.73±1.7	9	24.00	22.56±1.7
3	14.10	14.58±1.7	10	14.01	13.41±1.7
4	10.04	8.12±1.7	11	12.00	11.39±1.7
5	14.08	14.52±1.7	12	14.00	13.55±1.7
6	27.00	28.36±1.	13	13.00	11.80±1.7
7	10.08	9.84±1.7	14	14.02	13.90±1.7

Table 4.1.6. a-c Shows the distribution of error percentage in the estimated age for 43 upper first premolar teeth calculated from the control equation .

Table 4.1.6. a. The distribution of errors percentage in male and female

Range of Errors (years)	Percentage
0-1	95.35%
1-2	4.65%

Table 4.1.6. b. The distribution of errors percentage in male

Range of Errors (years)	Percentage
0-1	96.55%
1-2	3.45%

Table 4.1.6. c. The distribution of errors percentage in female

Range of Errors (years)	Percentage
0-1	71.43%
1-2	28.57%

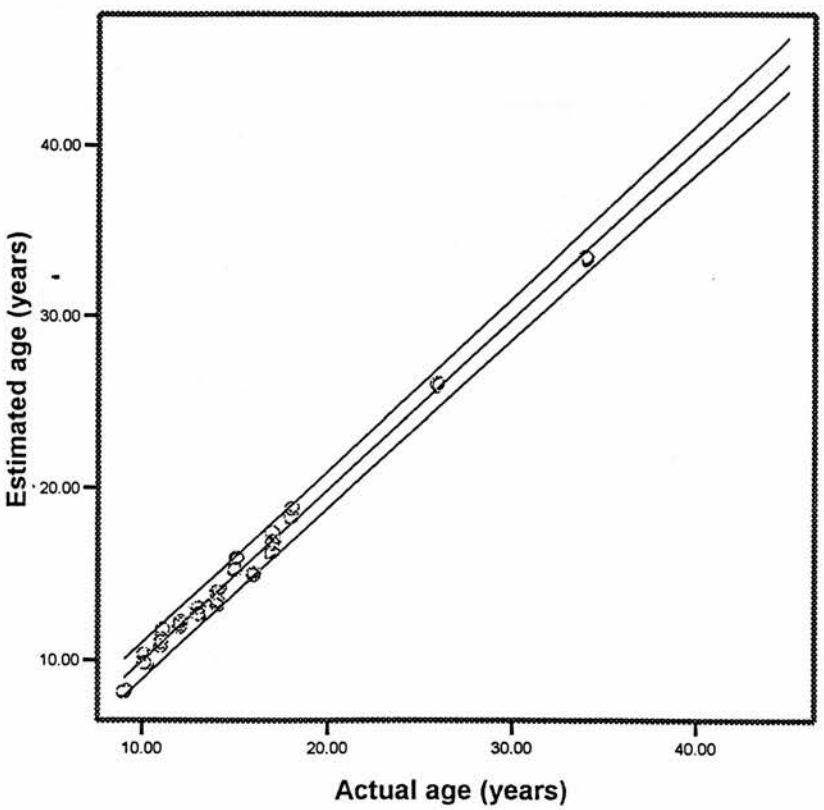

Figure 4.1.6.a The base line for Kuwaiti upper first premolar male and female teeth (n-43)

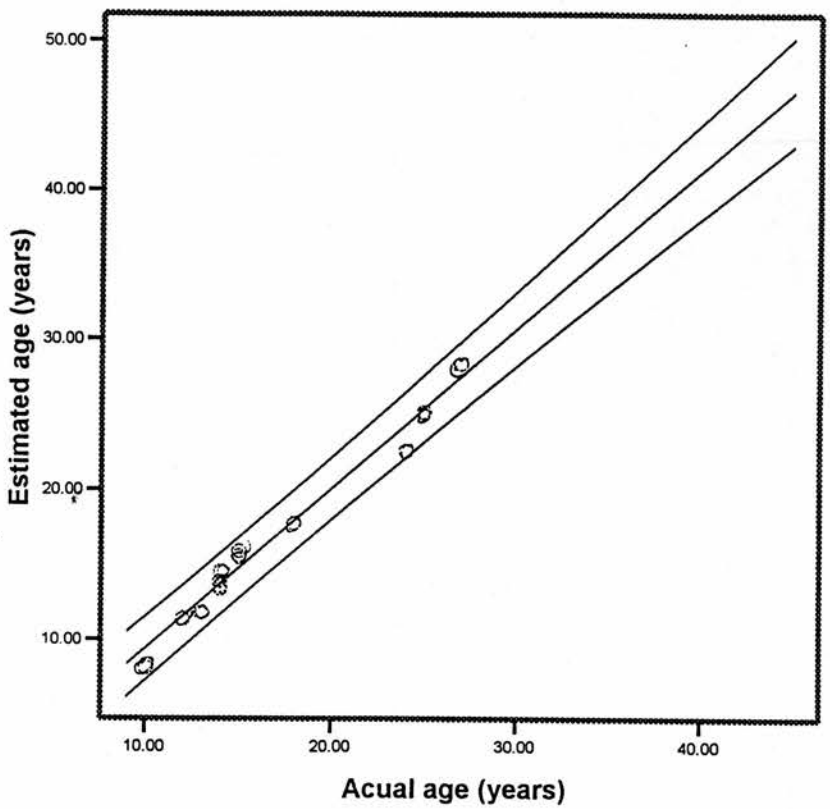

Figure 4.1.6.b The base line for Kuwaiti upper first premolar male teeth (n-29)

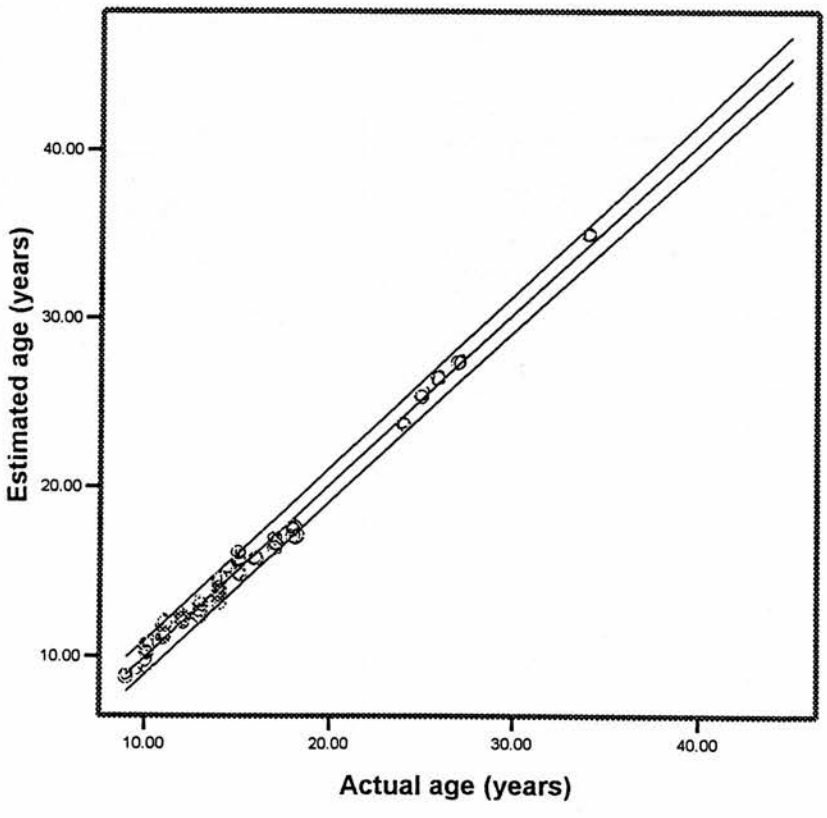

Figure 4.1.6.c The base line for Kuwaiti upper first premolar female teeth (n-14)

4.2. Age determination from teeth buried in the desert from D- and L- aspartic acid analysis at depths of 0.5M and 2.0M.

There were a total of 45 Kuwaiti first premolar teeth used for amino acid analysis, 14 of teeth whose age and gender were known to the writer were buried in soil at a depth of 0.5M (8 male and 6 female), the age ranged from 21.00 to 29.00 years. The 8 males, ranged from 21.00 to 27.0 years, and the 6 females age ranged from 22.0 To 29.00 years. Similarly 17 teeth were buried in soil at depth of 2.0M (10 male and 7 female). The age ranged from 19.0 to 29.00 years. For 10 males, the age ranged from 19.0 to 29.0 years, and the 7 females the age ranged from 22.0 to 27.0 years . This provided a total control group of 31 teeth,

The 6 teeth of recorded age and gender (but unknown to the writer at the time of the experiment) were buried in the soil at depth 0.5M and similarly 8 teeth of recorded age and gender (but unknown to the writer ate the time of the experiment) were buried in the soil at depth 2.0M thus providing a small test group of 14 teeth.

Table 4.2.1. Racemization of aspartic acid in 14 upper first premolar teeth buried in the soil at a depth 0.5m.

No	Sex	Age	D/L	No	Sex	Age	D/L
1	M	22.00	0.00242	8	F	22.00	0.00262
2	F	25.00	0.01434	9	M	21.00	0.00299
3	M	26.0	0.01450	10	M	25.00	0.00649
4	M	25.00	0.01050	11	M	27.00	0.02194
5	F	29.00	0.01157	12	M	24.00	0.00751
6	M	24.00	0.00411	13	F	23.00	0.00984
7	F	23.00	0.00428	14	F	25.00	0.00518

Table 4.2.2 and Figure 4.2.2. Shows the quantitative analysis ratio for 14 upper first premolar teeth buried in soil depth 0.5M. The linear regression and correlation coefficients (R square) were calculated for the relationship between the extent of aspartic racemization and actual age (t).

The relation between actual age and D/L ratio for 14 upper first premolar teeth buried in the soil at depth 0.5M.

$\ln[(1+D/L)/(1-D/L) = 0.00844\ t + (-0.07674)]$

$t=[(1+D/L)/(1-D/L) + 0.07674)/(0.00844)]$ .....(1)

R square = 52.0 %

The SE (standard error of estimation) was investigated by descriptive analysis:

SE = ± 2.3 years.

Table 4.2.2. An age determination for 14 upper first premolar teeth buried in the soil at a depth of 0.5m.

NO	Actual age	ln(1+D/L) / (1-D/L)	Error Years	NO	Actual age	Ln(1+D/L) / (1-D/L)	Error years
1	22.00	22.03	0.00	8	22.00	19.56	2.00
2	25.00	30.38	-5.00	9	21.00	22.23	-1.00
3	26.0	26.16	-2.00	10	25.00	23.42	1.00
4	25.00	24.80	2.00	11	27.00	28.71	2.00
5	. 29.00	27.83	1.00	12	24.00	23.77	2.00
6	24.00	22.60	1.00	13	23.00	26.23	-3.00
7	23.00	21.10	1.00	14	25.00	21.93	3.00

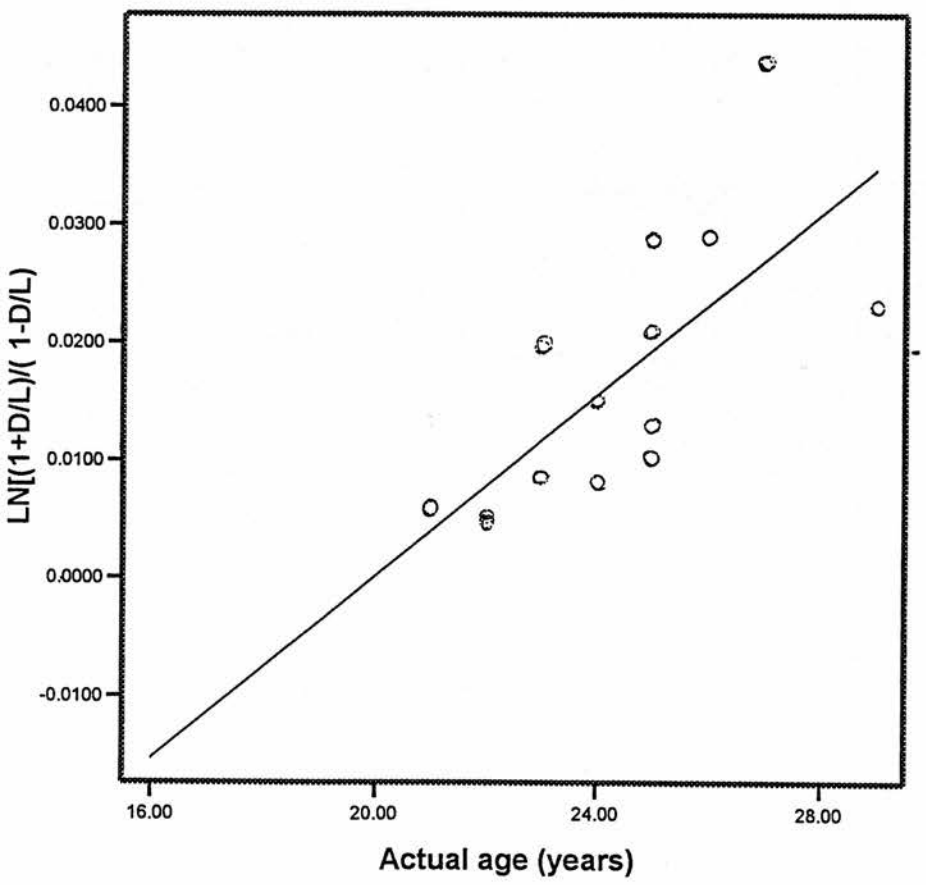

Figure 4.2.2. Relation between age and D/L ratio for both male and female (n = 14) in teeth buried at a depth of 0.5m.

Table 4.2.3. and figure 4.2.3. Shows the application of age estimation for the 6 upper first premolar teeth buried in the soil at depth 0.5M by using the calculated formula.

Estimated age for total M & F = [((1+D/L)/(1-D/L)+(0.07674)/(0.00844)]

The SE of control group was = ± 2.3 years

Known age = estimated age + SE (± 2.3 years).

Table 4.2.3. Age estimation of 6 upper first premolars buried at 0.5M

No.	Known Age Year	Estimated Age Year All Teeth
1	24.00	25.43 ± 2.3
2	25.00	27.07 ± 2.3
3	20.00	21.22 ± 2.3
4	21.00	22.34 ± 2.3
5	25.00	27.51 ± 2.3
6	20.00	21.52 ± 2.3

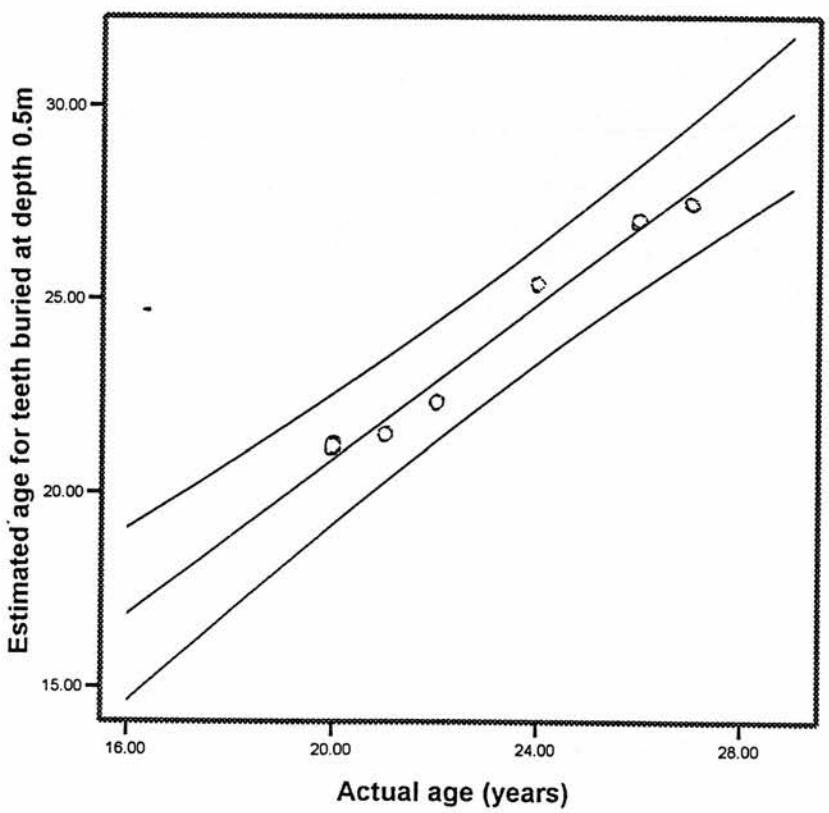

Figure 4.2.3. The base line for 6 upper first premolars (buried at a depth 0.5m) teeth both male and female (n = 6) in relation to known age

Table 4.2.4 and Figure 4.2.4 Shows the quantitative analysis ratio for 17 upper first premolar teeth buried in soil at a depth of 2.0M. The linear regression and correlation coefficients (R square) were calculated for the relationship between the extent of aspartic racemization and actual age (t).

Table 4.2.4 Racemization of aspartic acid in 17 upper first premolar teeth buried in the soil at a depth of 2.0M.

No	Sex	Age	D/L	No	Sex	Age	D/L
1	M	29.00	0.01366	10	F	22.00	0.00244
2	M	25.00	0.01017	11	M	20.00	0.00191
3	M	25.00	0.00457	12	F	25.00	0.01272
4	F	27.00	0.01579	13	M	26.00	0.00780
5	M	24.00	0.00485	14	F	27.00	0.02300
6	M	19.00	0.00130	15	F	27.00	0.02083
7	F	19.00	0.00755	16	M	28.00	0.01315
8	M	25.00	0.00559	17	F	26.00	0.01027
9	M	25.00	0.00751				

The calculated age of total dentine of 17 upper first premolar teeth buried in the soil at a depth of 2.0M.

$[\ln(1+D/L)/(1-D/L) = (0.00298)\ t + (-0.05418)]$

$t = [(1+D/L)/(1-D/L) + (0.05418)/(0.00298)]$ .....(1)

R square = 49.9 %

The SE (standard error of estimation) was investigated by descriptive analysis between known age and calculated age.

SE = ± 3.00 years.

For 10 Males

$t = [\ (1+D/L)/(1-D/L) + (0.0473)/(0.002503)$

R square 80.5% SE = ± 1.37 years

For 7 Females

t = [(+D/L)/(-D/L) + (0.0625)/(0.00360)

R square = 58.6% SE = ± 2.6 years

Table 4.2.4. Age determination of 17 upper first premolar teeth buried in the soil at a depth of 2.0M

NO	Actual age	ln(1+D/L) / (1-D/L)	Error Years	NO	Actual age	ln(1+D/L) / (1-D/L)	Error years
1	29.00	27.55	1.00	10	22.00	20.02	2.00
2	25.00	25.60	-0.6	11	20.00	19.67	0.30
3	25.00	21.45	3.00	12	25.00	26.92	-2.00
4	27.00	28.98	-2.00	13	26.00	23.61	2.00
5	24.00	21.64	2.00	14	27.00	33.82	-7.00
6	19.00	19.25	-0.30	15	27.00	32.36	-5.00
7	19.00	23.45	-4.00	16	28.00	27.21	0.70
8	25.00	22.14	2.00	17	26.00	25.27	0.70
9	25.00	23.42	1.00				

	Means of Known Age	Means of ln(1+D/L)/(1-D/L) Age	R square %	SE Years
Male	24.6	23.15	80.5 %	1.37
Female	24.7	24.41	58.6 %	2.6
Total	24.8	24.84	49.9 %	3.0

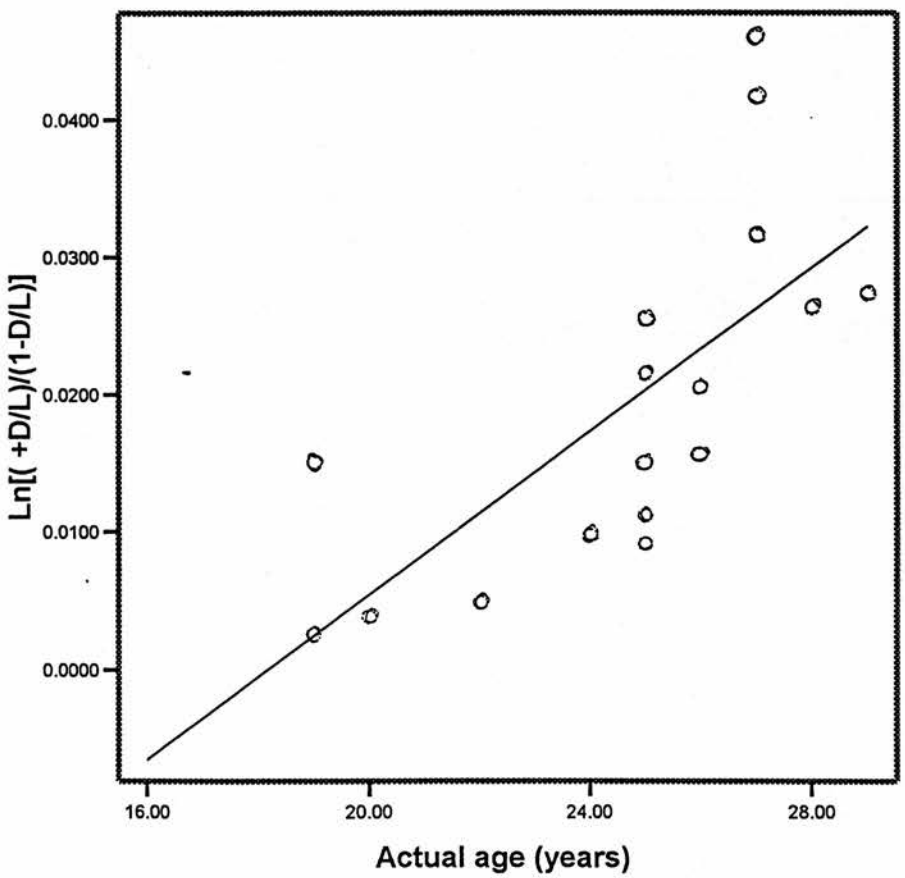

Figure 4.2.4. Extent of aspartic acid racemization in total dentine in both male & female (n = 17) in relation to known age in teeth buried at depth 2.0M.

Table 4.2.5. Shows the application of age estimation for 8 upper first premolar teeth buried in depth 2.0M by using the calculated formula .
Estimated age = [(1+D/4)/(1-D/4) + (0.07674)/(0.00844).

SE of control was = ± 3.0 years.

Actual age = estimated age + SE (± 3.0).

Table 4.2.5. Age estimation of 8 upper first teeth buried at a depth of 2.0M

No.	Know Age Year	Estimated Age Year
1	25.00	27.35 ± 1.37
2	24.00	25.40 ± 1.37
3	19.00	21.25 ± 1.37
4	26.00	28.78 ± 2.6
5	20.00	21.44 ± 1.37
6	18.00	19.05 ± 1.37
7	20.00	23.25 ± 2.6
8	19.00	21.94 ± 1.37

Table 4.2.6. Shows the quantitative analysis ratio for 31 upper first premolar teeth buried in at depths of 0.5 and 2.0M. The linear regression and correlation

coefficients (R square) were calculated for the relationship between the extent of aspartic racemization and actual age (t).

The relation between actual age and D/L ratio for 31 upper first premolar teeth buried in the soil at depths of 0.5 and 2.0M.

$\ln[(1+D/L)/(1-D/L) = 0.003247\ t + (-0.06142]$

$t=[(1+D/L)/(1-D/L) + 0.06142)/(0.003247)]$

R square = 50.1 %

The SE = ±2.60 years.

For Males

$\ln[(1+D/L)/(1-D/L) = 0.003248\ t + (-0.06368]$

$t=[(1+D/L)/(1-D/L) + 0.06368)/(0.003248)]$

R square = 61.3 %

The SE = ±2.06 years.

For Females

$\ln[(1+D/L)/(1-D/L) = 0.003169\ t + (-0.05639]$

$t=[(1+D/L)/(1-D/L) + 0.05639)/(0.003169)]$

R square = 43.5 %

The SE = ±2.35 years.

Table 4.2.6. Age determination of 31 upper first premolar teeth buried in the soil at depths of 0.5 and 2.0M

Buried teeth in both a depth 0.5& 2.0m			Not buried teeth		
Actual age	ln(1+D/L) / (1-D/L)	Errors Years	Actual age	ln(1+D/L) / (1-D/L)	Errors Years
22.00	20.39	1.61	16.00	15.79	0.23
25.00	27.75	-2.75	14.00	13.17	0.83
26.00	27.85	-1.85	15.00	15.38	-0.36
25.00	25.38	-0.38	12.00	12.19	-0.15
29.00	26.03	2.97	15.00	14.94	0.08
24.00	21.44	2.56	14.00	14.02	0.00
23.00	21.56	1.44	17.10	17.10	0.01
22.00	20.52	1.48	15.00	15.56	-0.48
21.00	20.76	0.24	15.00	14.56	0.44
25.00	22.92	2.08	11.00	11.33	-0.31
27.00	32.44	-5.44	26.00	26.18	-0.18
24.00	23.54	0.46	14.00	14.51	-0.43
23.00	24.98	-1.98	27.00	27.38	-0.38
25.00	22.12	2.88	15.00	15.62	-0.56
29.00	27.32	1.68	34.00	35.02	-1.02
25.00	25.54	-0.54	45.00	46.16	-1.16
25.00	21.72	3.28	20.00	20.49	-0.41
27.00	28.65	-1.65	18.00	17.42	0.62
24.00	21.90	2.10	14.00	13.47	0.53
19.00	19.72	-0.72	27.00	26.47	0.53
19.00	23.57	-4.57	19.00	19.31	-0.25
25.00	22.37	2.63	17.00	16.96	0.04
25.00	23.54	1.46	14.00	13.64	0.37
22.00	20.43	1.57	17.00	16.72	0.35
20.00	20.09	-0.09	23.00	22.53	0.47
25.00	26.74	-1.74	23.00	23.42	-0.42
26.00	23.72	2.28	18.00	17.67	0.38
27.00	33.08	-6.08	16.00	16.38	-0.36
27.00	31.76	-4.76	38.00	37.76	0.24
28.00	27.02	0.98	32.00	31.5	0.5
26.00	25.23	0.77	41.00	40.05	0.95

4.2.7 Effect of soil, temperature and pH on the D- and L- acspartic acid ratios in buried teeth at depths of 0.5M and 2.0M.

Table 4.2.7. a-c show the variation of D-and L ratio in dentine in teeth not buried and teeth buried at depths of 0.5M and 2.0M

Table 4.2.7.a The variation of D-and L-aspartic acid between teeth not buried and teeth buried at a depth of 0.5M

Not Buried		Buried 0.5m		p-value
No. of Teeth	Mean D/L	No. of Teeth	Mean D/L	
53	0.01885	14	0.00845	**

** = Significant at (P<001)

Table 4.2.7. b The variation of D-and L-aspartic acid between teeth not buried and teeth buried at a depth of 2.0M

Not Buried		Buried at 2.0m		p-value
No. of teeth	Mean	No. of teeth	Mean	
53	0.01885	17	0.00963	**

** = Significant at (P<001)

Table 4.2.7.c The variation of D-and L-aspartic acid between teeth buried at depths of 0.5M and 2.0M

Buried at 0.5m		Buried at 2.0m		p-value
No. of teeth	Mean	No. of teeth	Mean	
14	0.00845	17	0.00963	NS

NS = Not significant.

Table 4.2.8 Show the variation of D-and L ratio in dentine for female teeth not buried and female teeth buried at both depths of 0.5M and 2.0M

Table 4.2.8 The variation of D-and L-aspartic acid between female teeth not buried and female teeth buried

Not buried Female		Buried at 2.0m Female		p-value
No. of Teeth	Mean	No. of Teeth	Mean	
23	0.02499	13	0.01080	**

* * = Significant at (P<001)

4.3 Age determination of teeth from trace element analysis

A total of 553 teeth were used for trace elements analysis. 300 different teeth were not buried in soil (140 males and 160 females), the age range being 10.0 to 74.0 years. The male age ranged from 10.0 to 74.0 years. The female age ranged from 10.0 to 58.0 years. (control teeth)

The 53 first upper premolar teeth previously described (30 males and 23 females) were bisected vertically and one half used for amino acid analysis and the other half for trace element analysis. The age ranged from 10.0 to 45.0 years old. For 30 males, the age ranged from 10.0 to 27.0 years old, and for 23 females the age ranged from 10.0 to 45.0 years old.

The 100 different teeth of recorded age and gender but unknown at the time of the experiment to the writer were used for age and gender estimation from trace element analysis (Test).

Table 4.3.1 shows the values obtained for the trace element analysis for 300 different teeth. The linear regression and correlation coefficients (R square) were calculated for a relationship between the trace element analysis and actual age (t).

The calculated age of total dentine of 300 different teeth not buried in the soil.

The calculated formula for both male and female was:

t= [2.585 + 0.203* lnZn + 0.380* lnPb + 0.02965 lnMn-0.03194*lnFe-0.08494*lnSr0.189*lnMg + 0.03855*lnCu]

Calculated age = EXP(t) .....(1)

R square = 60.0 %

The SE (standard error of estimation) was investigated by descriptive analysis between known age and calculated age:

SE = ± 5.3 years.

The calculated formula for males was:

t=[2.736+0.177*lnZn+0.395*lnPb+0.01396*lnMn-0.03811*lnFe-0.126*lnSr-0.164*lnmg+0.01405*lnCu]

Calculated age = EXP(t) .....(2)

For 140 male

R square = 65.7 % SE = ± 5.47 years.

The calculated formula for female was:

t=[2.502+0.160*lnZn+0.382*lnPb+0.03353*lnMn-0.00868*lnFe-0.00401*lnSr-0.22lnMg+0.0706*inCu

Calculated age = EXP(t) .....(3)

For 160 female

R square = 63.1 SE = ± 5.4 years.

Table 4.3.1 Age determination for 300 different teeth

	Mean of Known Age	Mean of Calculated Age	R square %	SE Years
140 Male	23.54	23.09	65.7	5.37
160 Female	27.24	26.73	63.1	5.13
300 Total	25.51	25.03	60.0	5.24

Table 4.3.2 Shows the application of age estimation for 100 different teeth not buried in the soil by using the calculated formula .

The calculated formula for both male and female was:

t= [2.585 + 0.203* lnZn + 0.380* lnPb + 0.02965 lnMn-0.03194*lnFe-0.08494*lnSr0.189*lnMg + 0.03855*lnCu]

Estimated age = EXP(t)

The SE of control group was =± 5.3 years

Known age = estimated age + SE(± 5.3)

Table 4.3.2 The age estimation of 100 different teeth not buried

	Known Age	Estimated Age
100 Total M & F	25.20	24.26 ± 5.3

Table 4.3.3 Shows the standard error for 300 teeth (male and females) not buried in the desert according to age groups.

Age Groups (years)	Standard Errors
10-14	3.31
15-19	3.30
20-24	2.70
25-29	2.86
30-34	3.63
35-39	6.74
40-44	11.17

Table 4.3.4 Show the values obtained for the trace elements analysis for 53 upper first premolar teeth. The linear regression and correlation coefficients (R square) were calculated for a relationship between the trace elements analysis and actual age (t).

The calculated age for total dentine of 53 upper first premolar teeth not buried in the soil was:

t= [3.726-0.129*lnZn+0.407*lnPb+0.124*lnMn+0.02893*lnFe-0.01836*lnSr-0.197*lnMg+0.102*lnCu.

Calculated age = EXP(t).

R square = 60.9 %

The SE was investigated by descriptive analysis between known age and calculated age.

The SE= ± 5.39 years.

Table 4.3.4 The age determination of 53 upper first premolar teeth not buried in the soil

	Means of Known Age	Mean of Calculated Age	R square %	SE Years
Total 53 M & F	19.83	19.57	60.7	± 5.39

Table 4.3.5 Shows the comparison between trace element analysis and acid analysis for 53 first premolar teeth not buried in the desert.

	Mean of Known Age	Mean of Calculation Age	R square %	SE Years
Amino Acid Analysis	17.21	17.412	97.8	± 1.2
Trace Elements Analysis	17.2	17.4	60.9	± 5.39

Table 4.3.6 shows the values obtained for the lead analysis as an indicator of age for 300 different teeth. The linear regression and correlation coefficients (R square) were calculated for the relationship between the lead analysis and calculated age (t).

The calculated age of total dentine of 300 different teeth not buried in the soil.

The calculated formula for both male and female was:

t= [2.561 + 0.385* ln Pb]

Calculated age = EXP(t)

R square = 52.3 %

The SE (standard error of estimation) was investigated by descriptive analysis between known age and calculated age:

SE = ± 5.8 years.

Table 4.3.6 Age determination for 300 different teeth using lead only

	Mean of Known Age	Mean of Calculated Age	R square %	SE Years
300 Total	25.51	24.75	52.3	5.8

4.4 Age determination from trace element analysis in teeth buried in the soil

The distributions of 69 Kuwaiti third molar teeth were used for trace element analysis for age and gender determination.

19 of these teeth whose age and gender were known to the writer were buried in soil to a depth of 0.5M (12 males and 7 females) the age ranged from 18.0 to 36.0 years and similarly 16 teeth (10 males and 6 females) were buried at 2M, the age range from 16.0 to 35.0 years. This provided a control group of 35 teeth.

18 teeth of recorded age and gender (but unknown to the writer) were buried in a depth of 0.5M and similarly 16 teeth were buried in 2M thus providing a test group of 34.

Table 4.4.1. Shows the values obtained for trace elements analysis for 19 third molar teeth buried at a depth of 0.5M. The linear regression and correlation coefficients (R square) were calculated for the relationship between the trace elements and calculated age (t).

The calculated age of total dentine of 19 third molar teeth buried in the soil at depth of 0.5M.

The calculated formula for both male and female was:

t=2.538+004111*ln-0.112*lnPb-0.165*lnMn+0.153*lnFe+0.141*lnSr-0.295*lnMg+0.0720*lnCu.

Calculated age = EXP(t)----------1

R square = 49.2 %

The SE (standard error of estimation) was investigated by descriptive analysis between known age and calculated age.

SE = ± 3.0 years.

Table 4.4.1 Age determination of 19 third molar teeth buried at 0.5M

Total Teeth	Means of Known Age	Means of Calculated Age	R square %	SE Year
19	26.1	25.9	49.2 %	± 3.0

Table 4.4.2. Shows the application of age estimation for 18 different teeth buried in the soil at depth 0.5M by using the formula described above.

The calculated formula for both male and female was:

t=2.539+0.04111*lnZn-0.112*lnPb-0.165*lnMn+0.153*lnFe+0.141*lnSr-0.295*lnMg+0.0720*lnCu.

Calculated age = EXP(t).

The SE of control group was = ± 3.0 years.

Actual age = estimated age + SE (± 3.0).

Table 4.4.2 Age estimation of 18 third teeth buried at a depth of 0.5M

Total Teeth	Mean of Known Age	Mean of Estimated Age
18	23.1	24.7±3.0

Table 4.4.3. Shows the values obtained for the trace elements analysis for 16 different teeth buried at depth 2.0M. The linear regression and correlation coefficients (R square) were calculated for relationship between the trace elements and calculated age (t).

The calculated age of total dentine from 16 third molar teeth buried in the soil at a depth of 2.0M.

t=1.791+0.229*lnZn-0.03045*lnPb-0.213*lnMn+0.122*lnFe+0.112*lnSr-0.310*lnMg+0.06812*lnCu.

Calculated age = EXP(t).

With R square = 45.5 %

The SE (standard error of estimation) was investigated by descriptive analysis between known age and calculated age.

SE = ± 3.3 years.

Table 4.4.3. Age determination of 16 third molar teeth buried at a depth of 2.0M.

Total Teeth	Means of Known Age	Mean of Calculated Age	R square %	SE Year
16	26.50	26.39	45.5 %	± 3.3

Table 4.4.4. Shows the application of age estimation for 16 different teeth buried in the soil at a depth of 2.0 by using the calculated formula .

The calculated formula for both male and female was:

t=1.791+0.229*lnZn-0.03045*lnPb-0.213*lnMn+0.122*lnFe+0.112*lnSr-0.310*lnMg+0.06812*lnCu.

Calculated age = EXP(t).

The SE of control group was = 3.3 years.

Actual age = estimated age + SE (± 3.3)

Table 4.4.4. Age estimation of 16 third molar teeth buried at a depth of 2.0M

	Means of Known Age	Means of Estimated Age
Total	22.4	22.0 ±3.3

4.4.5 Effect of soil, temperature and pH on trace elements in dentine.

Table 4.4.5 a-c shows the variation in the mean value for various trace elements in dentine by gender for teeth not buried in the soil, teeth buried at depth 0.5M and at depth 2.0M.

Table 4.4.5 a Variation in the mean of trace elements in teeth not buried

	Teeth Not Buried		
	Male	Female	P-value
Zn	152.31	157.66	Ns
Pb	6.69	5.83	Ns
Mn	0.61	0.61	Ns
Fe	30.06	26.8	Ns
Sr	320.50	278.86	Ns
Mg	7.27	6.78	Ns
Cu	0.11	0.10	Ns

Ns = Not Significant

Table 4.4.5.b. Variation in the mean of trace elements teeth buried at a depth of 0.5M

	Teeth Buried at Depth 0.5M		
	Male	Female	P-value
Zn	110.59	107.32	Ns
Pb	5.28	2.91	**
Mn	0.47	0.36	Ns
Fe	25.05	10.77	**
Sr	572.10	466.62	Ns
Mg	7.73	7.50	Ns
Cu	0.160	0.151	Ns

** = Significant (P<0.001)

Ns = Not Significant

Table 4.4.5.c. Variation in the mean of trace elements in teeth buried at a depth of 2.0M

	Teeth Buried at Depth 2.0M		
	Male	Female	P-value
Zn	129.79	97.80	**
Pb	4.28	4.30	Ns
Mn	0.476	0.356	Ns
Fe	20.07	11.13	Ns
Sr	529.46	566.68	Ns
Mg	7.29	7.61	Ns
Cu	0.159	0.143	Ns

** = Significant (P<0.001)

Ns = Not Significant

Table 4.4.6.a-c shows the variation in mean for males in teeth buried and not buried at 0.5M and 2.0M.

Table 4.4.6.a. Variation of mean in male teeth not buried and buried at 0.5M

	Male Not Buried	Male Buried at 0.5m	P-value
Zn	152.31	110.59	**
Pb	6.69	5.28	Ns
Mn	0.606	0.472	**
Fe	30.06	25.05	Ns
Sr	322.50	572.10	**
Mg	7.27	7.73	Ns
Cu	0.1117	0.160	**

** = Significant (P<0.001)

Ns = Not Significant

Table 4.4.6.b. Variation of mean in male teeth not buried and buried at 2.0M

	Male Not Buried	Male Buried at 2.0m	P-value
Zn	152.31	129.79	**
Pb	6.69	4.28	**
Mn	0.616	0.476	Ns
Fe	30.06	20.07	Ns
Sr	320.50	529.46	**
Mg	7.27	7.29	Ns
Cu	0.1117	0.159	Ns

** = Significant (P<0.001)

Ns = Not Significant

Table 4.4.6.c. Variation of mean in male teeth at 0.5M and 2.0M

	Male Buried at 0.5m	Male Buried at 2.0m	P-Value
Zn	110.59	129.79	Ns
Pb	5.28	4.28	Ns
Mn	0.472	0.476	Ns
Fe	25.05	20.07	Ns
Sr	572.10	529.46	Ns
Mg	7.73	7.29	Ns
Cu	0.160	0.159	Ns

** = Significant (P<0.001)

Ns = Not Significant

Table 4.4.7. a-c Shows the variation of mean for female in teeth not buried and buried at depth 0.5m and 2.0m.

Table 4.4.7.a Variation of mean in female teeth not buried and buried 0.5M

	Female Not Buried	Female Buried at 0.5m	P-value
Zn	157.66	107.32	**
Pb	5.83	2.91	**
Mn	0.613	0.368	**
Fe	26.80	10.77	**
Sr	278.8	466.62	Ns
Mg	6.79	7.50	Ns
Cu	0.104	0.151	Ns

** = Significant (P<0.001)

Ns = Not Significant

Table 4.4.7.b Variation of mean in female teeth not buried and buried at 2.0M

	Female Not buried	Female Buried at 2.0m	P-value
Zn	157.66	97.80	**
Pb	5.83	4.30	Ns
Mn	0.613	0.356	**
Fe	26.80	11.13	**
Sr	278.8	566.68	**
Mg	6.79	7.61	**
Cu	0.104	0.1433	Ns

** = Significant (P<0.001)

Ns = Not Significant

Table 4.4.7.c Variation of mean in female teeth buried at 0.5M and 2.0M

	Female Buried at 0.5 M	Female Buried at 2.0m	P-value
Zn	107.32	97.80	Ns
Pb	2.91	4.30	Ns
Mn	0.368	0.356	Ns
Fe	10.77	11.13	Ns
Sr	466.62	566.68	Ns
Mg	7.50	7.61	Ns
Cu	0.151	0.1433	Ns

Table 4.4.8 shows the correlation coefficients between the trace elements and age in teeth not buried in the soil by gender.

Table 4.4.8. The correlation coefficients between the trace elements and age in teeth not buried in the soil by gender

	Teeth not Buried in the Soil			
	Male	P-value	Female	P-value
Zn	0.449	**	0.230	**
Pb	0.750	**	0.808	**
Mn	0.134	Ns	-0.102	Ns
Fe	-0.040	Ns	-0.206	**
Sr	-0.190	*	0.006	Ns
Mg	-0.004	Ns	-0.195	*
Cu	0.142	Ns	0.245	**

** = Significant (P<0.001)

Ns = Not Significant

Table 4.4.9 Shows the correlation matrix between the elements in male teeth not buried. According to the correlation matrix, statistically significant positive correlations(P<0.001) were obtained for Zn and Pb, Mn and Fe, Mg and Cu, Mg and Sr.

Table 4.4.9 Correlation matrix for elements in male teeth not buried

Zn	Zn	Pb	Mn	Fe	Sr	Mg
Pb	0.151**					
Mn	-0.014	0.046				
Fe	0.011	-0.094	0.320**			
Sr	-0.019	-0.035	-0.020	-0.123*		
Mg	0.005	0.063	0.251**	-0.115*	0.179**	
Cu	0.021	0.099	0.175**	0.082	0.054	-0.01

** = Significant (P<0.001)

Table 4.4.10 Shows the correlation matrix between the elements in male teeth buried at a depth of 0.5M. According to the correlation matrix, statistically significant negative correlations (P<0.001) were obtained for Zn and Mg.

Table 4.4.10 Correlation matrix of elements in male teeth buried at a depth of 0.5M

Zn	Zn	Pb	Mn	Fe	Sr	Mg
Pb	0.066					
Mn	0.326	-0.200				
Fe	-0.345	0.103	0.461			
Sr	0.524	0.089	0.150	-0.275		
Mg	-0.709**	-0.254	-0.223	0.504	-0.410	
Cu	-0.414	-0.067	-0.421	-0.052	0.008	0.141

** = Significant (P<0.001)

Table 4.4.11 Shows the correlation matrix between the elements in female teeth buried at a dept of 0.5M. According to the correlation matrix, statistically significant negative correlations (P<0.001) were obtained for Mn and Sr.

Table 4.4.11 Correlation matrix of elements in female teeth buried at a depth of 0.5M

Zn	Zn	Pb	Mn	Fe	Sr	Mg
Pb	-0.694					
Mn	0.345	-0.041				
Fe	-0.104	0.676	0.458			
Sr	0.035	-0.096	-0.884**	-0.464		
Mg	0.055	0.094	-0.508	-0.012	0.539	
Cu	-0.533	0.384	-0.327	0.334	0.016	-0.181

** = Significant (P<0.001)

Table 4.4.12 Shows the correlation matrix between the elements in male teeth buried at depth 2.0m. According to the correlation matrix, statistically significant negative correlations (P<0.001) were obtained for Zn and Cu.

Table 4.4.12 Correlation matrix of elements in male teeth buried at a depth 2.0M

Zn	Zn	Pb	Mn	Fe	Sr	Mg
Pb	0.44					
Mn	0.450	0.089				
Fe	0.133	0.424	0.588			
Sr	0.150	0.197	-0.045	0.076		
Mg	-0.200	-0.123	-0.268	0.283	-0.033	
Cu	-0.78**	-0.171	-0.536	-0.198	0.339	0.255

** = Significant (P<0.001)

Table 4.4.13 Shows the correlation matrix between the elements in female teeth buried at depth 2.0m. According to the correlation matrix, statistically significant negative correlations (P<0.05) were obtained for Mg and Cu.

Table 4.4.13 Correlation matrix of elements in female teeth buried at a depth of 2.0M

Zn	Zn	Pb	Mn	Fe	Sr	Mg
Pb	-0.490					
Mn	-0.127	-0.266				
Fe	-0.079	-0.119	0.524			
Sr	0.631	0.124	-0.655	-0.460		
Mg	0.647	-0.637	-0.302	-0.245	0.615	
Cu	-0.495	0.525	0.058	0.526	-0.504	-0.835*

** = Significant (P<0.001)

4.5 Gender determination for teeth not buried in the soil

4.5.1 Gender determination from D-and L-aspartic acid analysis of teeth not buried in the soil

Table 4.5.1 shows the sex estimation of 43 Kuwaiti teeth not buried in the soil. The results in this table show that there were 20 males (69.0%) and 6 females (42.9%) that were classified correctly.

The gender estimated equation is:

Y=-2.501+120.067*ln[(1+D/L)/(1-D/L)] for male.

Y=-3.166+140.424* ln[(1+D/L)/(1-D/L)] for female.

Table 4.5.1 The classification summary for 43 teeth not buried in the soil

	Predicted Group Membership		Total	Predicted %
	Predicted	Not predicted		
Males	20	9	29	69.0
Females	6	8	14	42.9

Table 4.5.2 Show the variation of D and L in dentine between male and female in teeth not buried in the soil.

Table 4.5.2. The variation of D- and L-aspatic acid between male and female in teeth not buried in the soil

Male		Female		p-value
No. of teeth	Mean D/L	No. of teeth	Mean D/L	
30	0.01413	23	0.02466	**

** = Significant at (P<001)

4.6 Gender determination in teeth buried at depths of 0.5M and 2.0M (From D- and L-aspartic acid analysis)

Table 4.6.1 shows the sex estimation of 6 Kuwaiti teeth buried in the soil at depth 0.5m. In this table there were 2 males (66.7%) and 2 female (66.7%) who were classified correctly.

The equation of sex determination:

Y=1.775+122.123*ln[(1+D/L)/(1-D/L)] for male.

Y=-1.438+101.312* ln[(1+D/L)/(1-D/L)] for female.

Table 4.6.1 The classification summary for 6 teeth buried in the soil at a depth of 0.5M

	Predicted Group Membership		Total	Predicted %
	Predicted	Not Predicted		
Males	2	1	3	66.7
Females	2	1	3	66.7

Table 4.6.2 Shows the sex estimation of 8 teeth buried in the soil at depth 2.0m. In this table there were 4 male (66.7%) and 1 female (50.0%) were classified correctly.

The equation for sex determination:

Y=-1.701+148.476* ln[(1+D/L)/(1-D/L)] for male.

Y=-3.672+255.299* ln[(1+D/L)/(1-D/L)] for female.

Table 4.6.2 The classification summary for 8 teeth buried at a depth of 2.0M

	Predicted Group Membership		Total	Predicted %
	Predicted	Not Predicted		
Male	4	2	6	66.7
Female	1	1	2	50.0

4.6.3 Gender determination from trace element analysis of teeth not buried in the soil

Table 4.6.3. shows the gender estimation of 100 teeth not buried in the soil by using the calculated formula from teeth not buried in the soil (control). 36 male (80.0%) and 42 female (76.4%) were predicted correctly.

For males :

-291.768+67.541*lnZn+1.163*lnPb-15.293*lnMn+12.066*lnFe
+23.256*lnSr+23.013*lnMg-8.748*lnCu

For females :

-291.083+68.676*lnZn+0.818lnPb-14.938*lnMn+11.642*lnFe
+22.598*lnSr+22.601lnMg-8.826*lnCu.

Table 4.6.3. The gender estimation of 100 teeth not buried in the soil

Sex	Calculation for males	Calculation for Females	Calculation for sex	Sex	Calculation for males	Calculation for Females	Calculation for sex
F	264.1	264.9	F	M	240.0	239.3	M
F	301.8	302.2	F	M	299.2	298.2	M
M	303.6	303.0	M	M	267.5	267.1	M
M	284.6	285.5	F**	F	320.3	320.3	M**
M	295.8	295.7	M	M	321.7	321.3	M
F	293.5	293.8	F	M	282.9	282.7	M
F	311.4	311.7	F	M	292.4	292.2	M
F	323.5	323.0	M**	F	265.2	265.1	M**
M	286.9	288.0	F**	F	255.1	254.6	M**
F	305.5	305.7	F	F	325.7	326.6	F
M	327.9	327.6	M	M	300.3	299.4	M
M	298.3	298.2	M	F	309.1	309.2	F
F	316.1	316.4	M	M	297.2	297.0	M
M	315.7	315.1	F	M	268.7	268.3	M
F	316.1	316.4	M	F	305.5	305.7	F
F	284.1	284.8	F	M	327.9	327.7	M
M	314.7	315.0	F	M	298.3	298.2	M
F	310.0	310.3	F**	F	316.1	316.4	F
F	282.9	283.6	F	M	315.7	315.1	M
F	309.0	309.1	F	F	316.1	316.4	F
M	293.9	293.5	F	F	284.1	284.8	F
M	292.7	292.3	M	M	314.7	315.0	F
F	296.1	296.3	M	F	310.1	310.3	F
F	298.3	298.5	F	F	282.9	283.6	F
F	309.9	309.5	F	F	309.0	309.1	F
F	294.8	295.2	M**	M	293.9	293.5	M
M	283.8	283.8	F	M	292.7	292.3	M
M	282.9	282.7	M	F	296.1	296.3	F
M	250.1	250.3	F**	F	298.3	298.5	F
F	319.0	318.6	M**	F	311.1	310.3	M**
F	301.2	300.9	M**	F	298.7	298.9	F
F	319.9	319.5	M**	F	278.6	278.7	F
M	289.7	289.7	M	M	280.8	280.8	F**

Sex	Calculation for males	Calculation for Females	Calculation for sex	Sex	Calculation for males	Calculation for Females	Calculation for sex
M	308.3	307.6	M	F	293.7	293.7	M**
F	298.0	297.1	M**	M	300.8	300.4	M
F	307.8	306.6	M**	M	308.0	307.8	M
M	299.1	298.2	M	F	252.1	252.8	F
F	258.3	259.3	F	F	314.6	314.7	F
F	259.1	260.1	F	M	285.3	284.8	M
M	283.5	283.5	M	F	297.5	297.0	M**
F	339.2	339.4	F	M	286.0	286.1	F**
F	339.2	339.4	F	M	283.4	283.4	F**
F	309.2	309.5	F	F	319.9	319.5	M**
F	326.6	326.9	F	M	289.7	289.7	M
F	327.8	328.1	F	M	281.8	281.6	M
M	235.5	233.9	F	M	288.1	288.2	F**
F	335.3	336.3	M	F	280.0	280.5	F
F	319.9	319.5	F	F	271.6	271.6	F
F	322.4	322.9	M	M	287.5	285.9	M
M	236.7	235.1	F	M	354.7	354.3	M

** Misclassified case.

Classification Summary

	Predicted Group Membership		Total	%
	Predicted	Not predicted		
Male	36	9	45	80.0
Female	42	13	55	76.4

Table 4.6.4 shows the sex estimation of 18 teeth buried in the soil at a depth of 0.5M. 11 male (100.0%) and 6 female (85.7%) were predicted correctly.

For Males :

-782.137-6.690*lnZn-44.947*lnPb-27.156*lnMn-40.520*lnFe

+106.896*lnSr+586.239*lnMg+50.134*lnCu

For Females :

-704.293+2.609*lnZn-38.301lnPb-24.352*lnMn-36.805*lnFe

+103.099*lnSr+520.143lnMg+43.042*lnCu.

Table 4.6.4 The classification summary for 18 teeth buried in the soil at a depth

of 0.5M.

	Predicted Group Membership		Total	Predicted %
	Predicted	Not Predicted		
Male	11	0	11	100.0
Female	6	1	7	85.7

Table 4.6.5 shows the sex estimation of 16 teeth buried in the soil at a depth of 2.0M. 10 male (100.0%) and 5 female (83.3%) were predicted correctly.

For Male :

-734.138+203.251*lnZn+14.757*lnPb+36.669*lnMn-13.763*lnFe

-8.952*lnSr+358.855*lnMg+65.131*lnCu.

For Female :

-686.113+184.134*lnZn+17.601lnPb+38.754*lnMn-16.035*lnFe

-7.123*lnSr+370.452*lnMg+59.553*lnCu.

Table 4.6.6 The classification summary for 16 teeth buried in the soil at a depth of 2.0M.

	Predicted Group Membership		Total	Predicted %
	Predicted	Not Predicted		
Male	10	0	10	100.0
Female	5	1	6	83.3

4.7. Soil analysis, temperature and pH

76 soil samples were dug according to a 3 x 3 Km pattern to investigate the soil properties, pH, and moisture.

The results of soil analyses showed these soils to be characterized by their calcareous nature. Their content of CaCo3 is higher than for all other soils in the area. These soils are generally non-saline, and their texture is coarse with generally low gravel content. The texture indicates a very low water-holding capacity. Very little gypsum is present in the great majority of samples.

The average pH of the 76-soil samples at a depth of 0.5M in the 3x3 Km area was 8.2 and 8.4 at 2.0 M.

The average soil moisture in 10 months in 1998 of 76-soil samples at a depth 0.5M was 14.8% , and 14.5% at a depth of 2.0 M.

The average concentration of trace elements in depth of 0.5M and 2.0M is summarized in tables 4.7.a-b.

Table 4.7.1.a. Soil analysis and pH in depths 0.5M and 2.0M

Soil Depth	PH	SO_4 (mg/l)	HCO_3 (mg/l)	CO_2 (mg/l)	NO_3 (mg/l)
0.5 m	8.2	3.33	1.84	N.D	0.20
2.0 m	8.4	1.30	1.44	N.D	0.055

Table 4.7.1.b. The mean concentration of trace elements in both depths (mg/kg)

Soil depth	Cu	Fe	Mg	Mn	Pb	Sr	Zn
0.5m	2.8	500	420	70	<0.5	30	6
2.0m	5.3	470	306	92	0.8	55	6.4

Temperatures

Climatological data for soil surface temperature from January 1998 to October 1998 were continuously recorded at Kuwait International Airport. A maximum temperature of 51.3^0C was recorded in August 1998 and a minimum temperature of 1.6^0C was recorded in January 1998.

Temperatures at both depths, 0.5M and 2M, were taken every hour by using an Omega automatic thermocouple from January 1998 to October 1998. Table 4.7.2.

Table 4.7.2. and Fig. 4.7.2 The mean soil temperatures at depths 0.5M and 2M

Month	Temp. at 0.5m (^{0}C)	Temp. at 2.0m(^{0}C)
January	16	18.6
February	17.7	19
March	19.9	20.6
April	25.7	25.1
May	32.3	30.3
June	37.3	34.8
July	39.7	37
August	40.9	38.8
September	39.2	36.7
October	36.4	34.9

The mean soil temperature at depths 0.5m and 2.0m in the 10 months in 1998 was 30.5^0C and 29.5^0C.

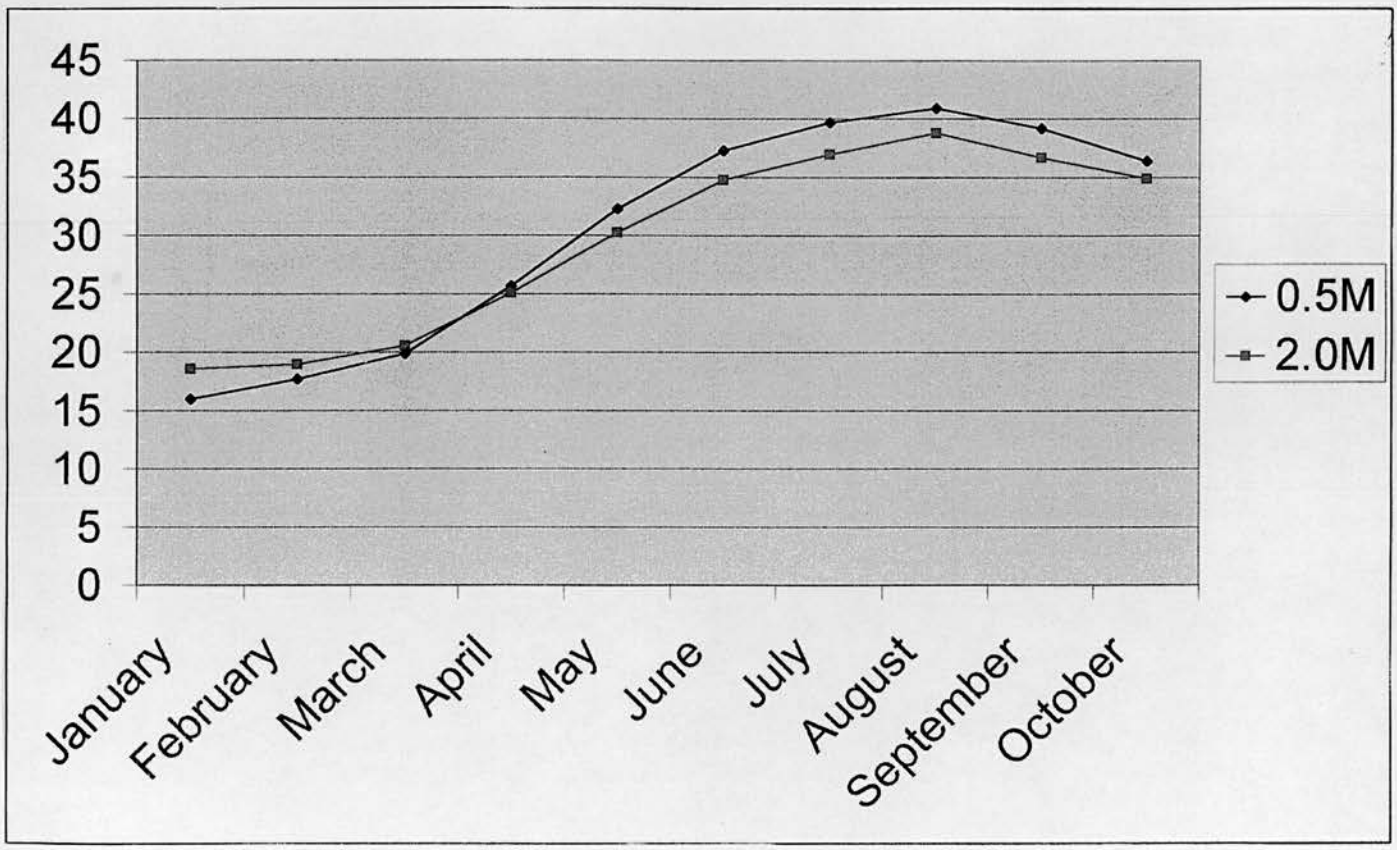

Figure 4.7.2 Shows the thermal Record from Kuwait.

Chapter 5

DISCUSSION

The results described in the previous chapter are examined here in the context of the original objectives of the project. Areas of potentially rewarding future work arising out of this research are suggested.

5.1 Problems associated with collecting teeth

The population of Kuwait which was reduced to 1-1.2 million people following the recent war with Iraq is composed of 48% Kuwaiti and 52% non-Kuwaiti people. The age group under 20 is estimated at 35-40% of the total population. National surveys are prerequisites for adequate planning and development of Health Services. Kuwait is one of the few countries in the Gulf region, where a large national Health Survey in 1986 (sample n=26.530) has been conducted. According to the Kuwait Health Survey [66,67], the mean DMFT was 11.7 for the whole adult population. The treatment of caries is still mainly by extraction and few fillings are placed. Almost two thirds of those examined had soft deposits, 45% had calculus, 46% intensive gingivitis, and 18% had advanced periodontal involvement. Thus, in addition to caries a fairly large percentage of the population had a serious periodontal problem. Among persons aged over 30 years, advanced periodontal involvement was found in 35-57% [59 ,60].

The teeth used in this research were extracted from living subjects of different ages. For these experiments they had to be extracted from healthy persons and free from restorations and dental caries. Because of the oral health problems in Kuwait, it was difficult to find teeth without caries (occlusal or cervical caries).

Kuwait is an Islamic country and it is illegal to take teeth from bodies at postmortem or dig in the cemetery for any experimental study. All these circumstances restricted the work to orthodontic treatment (i.e first upper premolar teeth and wisdom teeth). It

was difficult to examine an equal number of teeth from each group and I worked with the material at my disposal and I did the best I could in the circumstances.

5.2 Dentine and racemization

The first steps to positive identification of a cadaver include the determination of sex and age, race and stature. The assignment of age is therefore a crucial process and the estimate should be as accurate as possible.

Unfortunately, the traditional methods for aging adult cadavers are very subjective. If the body is in good condition the apparent age can perhaps be assessed visually but if the remains are degraded in any way morphological or histological aging techniques applied to bone or dental elements will be have to be used. Macroscopic and microscopic examination of adult teeth and bone have until recently provided an accuracy of at best +/- 3-6 years. [10,12,68,69,70,71,72,73,74].

Most living organisms produce amino acids solely of the L-configuration. These can racemize to D-forms over a lifetime but they are removed in tissues in contact with the circulatory system. However, in metabolically isolated tissues such as enamel, dentine . and intervertebral discs , accumulation of D-isomers can occur. The reaction has been suggested to play a role in the aging process of an animal [27 , 28].

The accumulation of D-aspartic acid during a lifetime has been used to age dentine [29 ,74]. The proteins in dentine are metabolically isolated and are held at a constant temperature (37°C ± 1°C) during life. Any degraded forms of the original proteins can therefore accumulate over time and the extent of degradation, (in this case racemization), can provide a means of age determination. Racemization of Asp in dentine has proved to be a good technique for determining age.

The accuracy of this technique can be excellent – where blind studies have been performed on teeth of known age, their ages have been estimated to within +/- 3 years of the actual age of the individual by two groups routinely performing the

analysis [30]. Not all groups have been so successful, perhaps because they have either used too few samples in the calibration curve or have too much variability of teeth. Furthermore, between laboratories there are significant differences in the reported rates of racemization. Theoretically, the same dentine sample from the same region of the teeth should give the same rate of racemization, so the inconsistencies may be due to differences in the methods used. Standardization of the technique is essential if it is to be used widely by the forensic community and be legally accountable. The construction of calibration curves with excellent correlation coefficients is of no use unless the technique can be applied with confidence to cases of aging unknown individuals in real forensic cases. The routine nature of the method means that many forensic laboratories could adopt it and there is a need for international standardization.

In forensic practice the coronal dentine may be largely destroyed or removed (caries, large dental fillings, crowns). but the root dentin is usually intact. In such cases it is neither possible to obtain coronal dentin nor "entire dentine of central longitudinal sections" in a reproducible manner (30). Ohtani and Yamamoto 1987 [52] found different values for the extent of aspartic acid racemization in crown and root of the same tooth.

In Kuwait there is a high incidence of tooth caries in adult individuals. We investigated total dentine and the acid soluble and acid insoluble dentine protein fractions of roots to ascertain whether age at death can also be determined on the basis of aspartic acid racemization in these cases in which only root dentin is available.

5.3 Sampling strategy

The type of teeth chosen for the analysis may influence the result of an age at death determination. This is because dentine age is not equivalent to chronological age – the age of tooth at extraction must be corrected by the length of time it takes the tooth to form. (51,75). This is different for each tooth; incisors form more quickly than premolars which form more quickly than premolars which form more quickly

than third molars. There would be few problems if the tooth correction standards used in all studies were the same, but this is not the case. Some studies do not correct the dentine ages at all (this may be acceptable if all the teeth are of the same type (15) although there may be biological variation between individuals. Also, it is not certain if the formation times represent the true age of the proteins we wish to analyze. Tooth formation standards are usually expressed as a range of years from the start of mineralisation to the completion of the crown and root, (75). These problems can be avoided if teeth of the same type are used, but ideally individual calibration curves should be constructed for each tooth type.

There have been many studies comparing racemization in different regions of dentine. They include comparisons of different areas of the crown, (54) –vertical section (47)– vertical, and horizontal section (76) – (lingual vs labial regions), primary and secondary dentine (77) and root and crown (14). The general consensus is that there is variation in rate within the dentine, particularly for the more recently formed secondary dentine which gives irregular results, (54,77). Ohtani, 1995b, (78) advocates the use of whole longitudinal sections rather than transverse ones, taken from the center of the tooth because the racemization varies from labial to lingual sides (76). Root dentine was found to give a faster rate than crown (15) and these authors concluded that it gave good age estimates, as did Mornstad et al, 1994 (32).

5.4 Influence of fixative

Many studies have used tooth specimens that have been stored in fixatives such as formaldehyde (,30,32,51,79). It is known that formaldehyde cross-links protein. [80]. These studies have attempted to investigate the influence of fixative on racemization in human dentine. Unfortunately, they have been unable to obtain reproducible results from the storage of dentine in formaldehyde and the data suggests that the amount of racemization induced during acid hydrolysis is altered in the acid-soluble extract of formaldehyde-stored dentine and therefore the use of formalin fixatives is not advisable. The use of formalin in some studies and not in others could account for some of the variations in racemization rates across studies, but this is an area which requires further investigation.

5.5 Bleach treatment

Instead of using formalin to fix teeth, some researchers have opted to remove any adhering soft tissue from the teeth using sodium hypochlorite. (54,56). They believe that this is a good method for inactivation of microorganisms in addition to removing soft tissue. However, there are concerns that prolonged exposure to bleach could oxidize and destroy some of the more labile soluble proteins in dentine. Again, the impact of bleach treatment upon the measurement of D/L value is something that requires further investigation.

5.6 Techniques used

This study was carried out to explore whether ordinary High Performance Liquid Chromatography (HPLC) technique could be used instead of the gas chromatographic (GC) technique, which needs expensive equipment and special columns. The results show that HPLC technique can be used to give a good result, even if most of the studies that have used GC technique seem to have a higher reliability, up to $r = 0.99$,vs $r = 0.97$. It is presently not known if the reason for this discrepancy is a difference in the techniques per se, or if the HPLC technique needs further refinements to make full use if its capacity. Van den Oetelaar et al. (81) compared the separation efficiency of capillary gas chromatographic and diastermeric high performance liquid chromatographic methods and found that the latter was preferred because of its higher reproducibility and convenience. Only the resolution was lower with HPLC technique.

The method has high sensitivity, and when small samples (less than 0.5mg. of dentine) are tested contamination with proteins from other sources, such as blood, pulp or periodontal tissues may significantly increase the relative amount of the L-form, giving a too low an estimate of the age. Also newly formed secondary dentine many decrease the relative amount of the D-form. Similarly, contaminated with

bacteria with D-form aspartic acid in their cell walls may cause an apparently too high an age (30).

5.7 Soil analysis in burial sites

The soil in Kuwait is characterized by sand and is poor in organic material in general. In 1969 the Food and Agricultural Organization (FAO)[82] classified the soil in Kuwait into four types:

Desert soil, 78% of Kuwait. Desert-Regosol Intergrade, 14% of Kuwait. Litho Soil, 1% of Kuwait and Alluvial Soil 7% of Kuwait.

There are two cemeteries in Kuwait, one in the south and the other in the north. The Kuwait Institute of Scientific Research geologist took soil samples from these cemeteries and they found that the two contained the same soil type. Small scale sampling as distinct from digging up a corpse was permitted.
The Geologist located for me an area containing soil similar to that found in the cemeteries. We buried the teeth in this desert soil at depths of 0.5M and 2.0M to simulate a criminal burial and an orthodox Islamic burial.

Soil samples were taken to find whether or not the soil varied in composition from one site to another in the same general area.. It was found that, there were only some minor differences in pH, moisture and trace element composition.

Soil samples were taken from depths ranging from 50 to 200 cm, using a soil auger. Visual observations for each soil samples were recorded in the field using a standard soil description sheet that depicted the presence, properties, thickness and depth of soil horizons. For each horizon or layer the following properties were tested in the field: wet and dry soil color using the Munsell chart, texture, structure, consistency, reaction to diluted HCl, presence of carbonates or gypsum, presence of gravel and presence of compacted layers or hardpans.

As shown by the results of the soil analyses these soils are characterized by their calcareous nature. Their content of $CaCO_3$ is higher than for other soils in the area. These soils are generally non-saline, and their texture is coarse with generally low gravel contents. The texture indicates a very low water-holding capacity. The analyses show no specific ion effect. Very little gypsum is present in the great majority of profiles.

pH is alkaline, ranging from 8.2 to 8.9 at depths of 0.5m and 2.0m.

The soil moisture was very low at the two depths during the dry season. It increased during late January and remained almost constant after that, until early April when it increased again before rapidly falling.

It should be noted here that the April increase is due to the fact that the sampling date followed the heavy rain of late March. The 0 – 15-cm depth showed a higher percent of moisture on all occasions. This could be explained due to the surface soil texture having a slightly better water holding capacity due to the small amount of humus it contains compared to the humus free and sandy deeper soil.

The average soil moisture at a depth of 0.5-m was 14.8% and 14.5% at a depth of 2.0 m.

Climatological data for soil surface temperature from January 1998 to October 1998 were recorded at Kuwait International Airport. A maximum temperature of 51.3°C was recorded in August 1998 and a minimum temperature of 1.6°C was recorded in January 1998.

Temperatures at both depths, 0.5M and 2M, were taken every hour by using a thermocouple from January 1998 to October 1998.

The maximum temperature was 40.9 °C and minimum was 16°C at depth 0.5m ; and 38.8'C and 18.6'C at a depth of 2.0m .

5.8 Age determination from D and L-aspartic acid analysis from teeth not buried in the soil

Age estimation from teeth is one of the best methods available for the determination of chronological age of individuals with uncertain birth records [5-14,83].

The racemization of amino acids is a reversible first-order reaction, and is relatively rapid in tissues in which metabolism is slow. The amino acids are incorporated into the tooth as L-enantiomers. However, with time, amino acids racemize with an increased ratio of D-enantiomers, metamorphosing into a racemate (84,85). Among the amino acids, aspartic acid shows a high reaction rate and is expected to provide useful information on changes occurring in living tissues with time.

A number of studies have shown the possibility of age estimation by aspartic acid racemization in both enamel [27] and dentine [29,34,51,79].

In this study High Performance Liquid Chromatography (HPLC) was used to estimate aspartic acid racemization in samples of total dentine derived from upper first premolar teeth. The regression equations used were those described by Bada and Schroeder (86 , 87) and Smith et al (88).

The results demonstrate that the HPLC technique can be used to provide good results with a correlation coefficient of 0.978 and an estimated error of +/- 1.2 years in calculating actual age.

This is very comparable with results published by Mornstad et al in 1994 (32) who demonstrated a correlation coefficient of r= 0.95 and an estimated error of +/- 2 years.

The first objective of this research has therefore been achieved , namely the development in Kuwait of an accurate method of age estimation based upon the conversion of L- to D- aspartic acid using dentine from the middle third of the maxillary first premolar tooth.

It took a long time (two years) to achieve this objective since nobody in Kuwait was familiar with the process and I had to learn the technique in England , purchase and set up the apparatus and finally work with it until consistent results were obtained.

A possible explanation for apparently slightly better results described here compared to Mornstad's is that he used teeth exposed to formalin fixation. It is known that formaldehyde cross-links protein , particularly amino acid side chains (80).
The method has high sensitivity (less than 0.5mg of dentine being required as a substrate)), contamination with proteins from other sources, such as blood, pulp or periodontal tissues was avoided by the removal of any adhering tissues, from the teeth by using sodium hypochlorite [54,56]. Similarly there was no contamination with bacteria from caries by using caries-free teeth. This was important because contamination with these proteins might have significantly increased the relative amount of the L-form, giving a too low estimate of the age and in contrast contamination with bacteria with D-form aspartic acid in their cell walls might have caused a too high age estimate of age [32].

According to Helfman and Bada ,1976[27] and Ogino et al, 1985 [51], the extent of aspartic acid racemization in dentine depends upon the age of the dentine, which cannot be equated exactly with the age of the individual. The difference between individual age and dentine age varies and is dependent upon which tooth is analysed since teeth form at different ages. However the steady conversion rate from L to D enamoters in life means that provided the type of tooth is known age associated charts for that defined tooth can be created and used to estimate the age of an individual provided comparable samples are taken from the same tooth type. Ie it is necessary to compare the L/D ratios in an upper first premolar with the graph of L/D ratios experimentally created from studies of upper first premolars from people of known ages.

Ogino et al 1985 [51] also recognized that a separate consideration of individual tooth types might lead to a more accurate age estimation by averaging results for the different teeth. This is plausible but I did not have enough materials to carry out such a study.

In forensic practice the coronal dentine may be largely destroyed or removed (caries, large dental fillings, crowns) whereas the root dentine is usually intact. In such cases it is neither possible to obtain coronal dentine nor "entire dentine of central longitudinal sections" in a reproducible manner. Since Ohtani and Yamamoto (30,52) found different values for the extent of aspartic acid racemization in crown and root of the same tooth, the published results for coronal dentine or the "entire dentine of central longitudinal sections" (29,51,52,) cannot be directly applied to root dentine. Ohtani and Yamamoto 1987 (52) investigated exclusively total root dentine in a small number of teeth. Based on their investigations no conclusions can be reached on the applicability of the method for root dentine. Mornstad et al. 1994 (32) give an explanation for a lower correlation coefficient in their study using in the same experiment different types of teeth. It was discovered that the teeth contained microorganisms producing enzymes capable of promoting racemization (55) when they used teeth with coronal caries in their experiment.

Moreover, results of Ohtani and Yamamoto, 1987 (52). 1991(30) showed that there are different rates of aspartic acid racemiztion in coronal and root dentin in the same tooth. Values for the extent of aspartic acid racemiztion in coronal dentin tend to be higher that the corresponding values for root dentin in young teeth, whereas the ratio appears to be reversed with increasing age . The ambient temperature of crown and root may by different, resulting in different racemization rates. It has been shown that there is a heat flow from the periodontal tissues to the tooth surface and a gradient of up to 37 C has been reported [53]. Breathing, talking, sipping cold or hot drinks etc. could contribute further to such thermal differences. The actual magnitude of such effects is unknown, but small fluctuations in temperature can bring about significant changes in the rate of racemization as demonstrated in this work following burial of teeth in a warm environment.

Most authors have investigated coronal dentin only (15,51). Ohtani 1995 [14] divided the lower central incisors into four blocks and they found that the coefficient of correlation between the D/L ratio and true age varied only slightly among the blocks. The correlation coefficient was higher in longitudinal section ($r = 0.995$) than in transverse sections. In transverse sections, the error between true and estimated

age was relatively marked in the middle- advanced age groups. In longitudinal sections, the error was ± 3 years . The D/L ratio of amino acids is higher in older tissue . As dentine forms from the crown toward the root apex, the D/L ratio should be higher in the crown and decrease toward the root apex. These findings are consistent with the process of dentine formation [78]. Considering these results. The authors recommended the analysis of the "entire dentine of central longitudinal sections" [30,48,52].

5.9 Age determination of teeth buried at depths of 0.5M and 2.0M in desert soil.

In this experiment 14 upper first premolar teeth whose age and gender were known to the writer and 6 first premolar of recorded age and gender (but unknown to the writer at time of the experiment) were buried at a depth of 0.5M. A further 17 upper first premolar teeth (of known age and gender) and 8 upper premolar teeth of known age and gender (but unknown to the writer at the time of the experiment) were buried at a depth of 2.0M.

The results are interesting. The correlation coefficient of actual age with estimated (experimental) age for the teeth not buried was high ; r=97.8% but low for teeth buried at a depth of 0.5M ; 52.0% . Similarly the correlation coefficient for teeth buried at 2.0M was only 49.9%.
The standard errors of age estimation were low in teeth not buried (1.2y) compared with teeth buried at 0.5M (2.3y) and 3.0y for teeth buried at 2.0M

In this experiment it was also found that there was a significant difference between the D/L ratio in teeth not buried and the D/L ratio in the teeth buried at a depth 0.5M, a significant difference between the D/L in teeth not buried and the D/L ratio in teeth buried at a depth 2.0M, but there was no significant difference between the D/L ratio in teeth buried at a depth 0.5M and teeth buried at a depth of 2.0M.

This data requires close scrutiny. Initially the laboratory techniques were learnt in the laboratory of Dr Matthew Collins , University of Newcastle , England and then developed in Kuwait.

Once the methodology appeared to be delivering consistent results it was necessary to test it more rigorously and to this end a total of 96 upper premolar teeth were analysed. As recorded above an estimate of age was achieved with an error of only +/- 1.2 years from the known age. This is comparable with and a slight improvement upon the results published by Mornstad et al (32) who achieved +/- 2 years at a 95% confidence level similar to that achieved by Gillard et al four years previously (54).

While the test teeth were being collected and examined comparable upper premolars were left buried in the desert. Analysis of these teeth brought interesting results.

The standard error increased from 1.2 to 2.3 years and although this could have been due to experimental ,(laboratory) , error it seems unlikely since the analysis was carried out shortly after refining the technique with the control teeth and when the laboratory staff were performing consistently and on a daily basis. The factors to consider are temperature , dehydration ,pH and the influence of bacteria.

Temperature

Racemization continues after death and the rate of racemization appears to be largely temperature dependent for the speed of conversion from L-aspartic acid to the D-form decreases with falling temperature. The average temperature in all latitudes is below body temperature of 37'C. According to Ogino et al (51) a storage period of 10 years at 15'C results in an error of age estimation of merely 0.02 years using the analytical methods used in the current study.

Comparison with Ogino et al's work (51) and this study reveals that they analysed 14 cadavers (contrast with 45 buried specimens in this work) and assessed the age at death by the use of aspartic acid racemization in dentine. All the cadavers were later identified revealing their actual ages. In their publication they state , " no significant correlation can be determined on discrepancy between the estimated and actual ages and such factors as the cause of death and the amount of time that elapsed since

death" Thus it would appear that while racemization may continue after death the rate at 15'C is extremely slow. Masters (13) makes a similar observation.

Ohtani (89) , Bada et al (6), King et al (84) and Schroeder et al (90) have all demonstrated in the laboratory that the D/L ratio for racemization of aspartic acid in dentine increases as the temperature increases and time elapses. This phenomena was attributed to activated and enhanced racemization in direct response to an elevation in temperature.

However a valuable contribution to the literature by Masters (13) suggests that the relationship of racemization rate to temperature may be quite complex. In Master's study aspartic acid racemization was studied in dentine from six persons at autopsy where independent age information was available. These six cadavers represented a range of fates including recent demise , burial and ground surface exposure. Aspartic age at death estimates were identical in five of the six cases within the error of known dental age. In one case however the racemization was inflated by 10 years. This sample came from a partly skeletonised corpse which had been lying exposed on the ground for a period of 51 days in February and March in San Diego , California , USA.. The ground level temperature at this time of the year averages between 9' and 18'C (Encyclopedia Britannica) but with average annual rainfall of only 50. cms direct sunlight levels and radiant heat may be significant but this data for the site where the corpse was found is not provided. If this greatly enhanced racemization is correct and not an aberration then it might be due to "bursts" of racemization caused by periods of relatively intense radiant heat . However other factors need to be considered. For example desiccation slows racemization and bacteria may enhance it (see below).

A single result such as this published by Masters requires to be noted but clearly requires substantiating.

Experimentally racemization at higher temperatures is observed to be highly temperature sensitive. Bada (91) has suggested that the rate of racemization of aspartic acid at 150'C should be 800,000 times faster than at 37'C which calculates at adding 92 years to the estimated age if a temperature of 150'C is sustained for one

hour. The work of Ohtani et al in 1989 (92) supported this claim by showing that age estimation by racemization was not reliable in fire victims. However more recently in 1997 (93) Ohtani's team have provided encouragement for the wider use of aspartic acid racemization age estimation in fire victims by noting that if only the soluble protein fraction is analysed the rate does not appear to change following heating to 150'C for one hour and these authors conclude that age calculated from the D/L ratio of dentinal soluble peptides from burnt bodies is sufficiently accurate as to be used as a legitimate method of age estimation.

The experimental results described in this Thesis show a standard error of +/- 2.3 years for the age of the buried teeth compared to the unburied teeth. Ie the results are more variable. The thermocouple readings show that over the ten month trial period the mean soil temperature was 30.5'C at 0.5M and 29.5'C at 2M but associated with a range of temperature from 16 – 40.9'C at 0.5 M and 18.6 – 38.8'C at 2.0M. At a depth of 0.5M the temperature was at or slightly above body temperature (36.4'C – 40.9'C) for five months and only slightly lower (34.8'C – 38.8'C) at 2.0M. The differences in racemization between the two depths was insignificant but was significantly different from that in unburied teeth. On examination of the thermocouple graph before analysing the data I anticipated that the amount of racemization would be greater and was disappointed to find that the standard error being 2.3 years precluded stating this. Careful examination of the figures however reveals that in 28 of the 45 buried teeth the amount of racemization was increased compared to 17 in which it appeared to be less. Interestingly when the test teeth results are examined separately , as compared to buried control teeth , it can be seen that all 14 , without exception , showed increased racemization. These were the last teeth to be analysed and the code was not cracked until after the calculations had been made.

It is a fault in my experimental design that these teeth were not coded inclusively within the whole batch of buried teeth since now the question must arise – was the analysis of these test teeth more carefully carried out than the control teeth ? I do not think so but the result suggests an experimental error.

Alternatively since these were the last teeth analysed is it possible that we were still improving our technique. ?

However the evidence further supports the statement that the rate of racemization is partially temperature dependent and that in estimating age from bodies buried in the ground a knowledge of the soil temperatures and the length of time exposed to these temperatures is critical.

At the time of writing this Thesis this is the first attempt to measure soil temperatures at burial over 24 hour periods for a substantial period of time (10 months) and to note the remarkable consistency between the temperatures in a shallow grave compared to a deep grave , at least in the Middle East.

The high average temperature in the shallow grave was a surprise to my UK Supervisors but not to my colleagues in Kuwait. The Bedouin teach their children that to survive the very cold surface temperatures that can be experienced at night in the desert they need only scrape a shallow depression in the sands and the sands , heat-soaked by day , will keep them warm.

In forensic matters knowledge of these shallow grave temperatures is obviously now important and extrapolation of results derived from experiments in temperate climates are not necessarily applicable anywhere else. This work I am describing needs to be repeated in different climatic conditions and for longer periods in order to more fully appreciate the effects of temperature and burial for it would appear that the accuracy of the technique depends largely upon minimizing exogenous temperature effects

Dehydration

It is known that dehydration does slow the rate of racemization (94, 95) as spectacularly witnessed by the absence of D- amino acids in fossilized insects entombed in amber (96). However the pore size distribution of mineralised collagen , which is dominated by pores less than 10nm (97) and the tight hydration shells of collagen itself (98) means that it is not possible to remove all water by desiccation.

In preliminary work (as described above Chapter 4.7) 76 soil samples were taken and it was found that average soil moisture in the 10 month burial period in 1998 was 14.8% at a depth of 0.5M and 14.5% at a depth of 2.0M. The percentages describe

the weight difference in soil before and after desiccation in the laboratory. These percentages are low and according to local geologists are to be expected since the soil coarse with a low water-holding capacity.

I do not have any soil samples from a temperate climate nor from other soil types but in my view this low water content and relatively high temperature at depth would probably combine to provide a desiccating situation and further studies on buried teeth weighed before and after a 10 month period at these depths might establish whether or not this opinion is valid. If it were found to be the case then desiccation might partly explain why some teeth provided an age estimation less or very similar to the known age and hence provided results which contributed to the increased standard error for age estimation in the buried teeth. It should be noted , however , that burying teeth in the manner described in this Thesis is quite artificial and teeth protected by bone in a local environment modified by decaying or mummified flesh may be much less desiccated. This is not known and experiments with buried animal heads might be informative. If desiccation were reduced then the effects of a warm microenvironment at burial might be enhanced.

PH and humidity

As early as 1972 and 1975 Bada et al (86 ,87, 99)) had studied the influence of pH on the rate of racemization of aspartic acid and found as was later confirmed by Ohtani et al in 1995 (78) in their studies on aspartic acid in dentine that the rate of racemization increases as a function of pH. Ohtani also published in 1995 (100) work which demonstrated that when teeth were left in a dry environment for a year their estimated age increase was negligible and that in an environment of pH 4 extremely small ; 0.1 years. This compares and is consistent with the findings of Yoshinage et al earlier in 1990 (101) who had shown that specimens left in a environment of pH 9 for I year aged 0.6 years and those left for 5 years in this environment aged 3.2 years. However Ohtani (78) also observed that the findings in dentine were not consistent with findings in bone by Bada and Shou (102) who had found no relation between racemization of aspartic acid and pH and clearly the matter is not simple.

A possible explanation offered by Bada is that calcium hydroxyapatite in bone itself acts as a buffer against variations in pH.

The work described in my study is consistent with the findings of Ohtani in that the teeth in this study were buried in a soil pH 8.2 – 8.4 for ten months and the process of racemization appears , for most teeth to have continued . The possible buffering capacity of calcium hydroxyapatite in dentine has not be assessed in my work and further investigations are required.

Bacteria

Gillard et al in 1990 (54) demonstrated that teeth from people of known age in the Spitalfields crypt displayed aspartic acid racemization ages which were too old for the individuals. It was discovered that the teeth examined contained microorganisms producing enzymes promoting racemization (55) and this may have been the cause of the discrepancy. More recent studies in 1997 (56) have met with no more success with Victorian teeth of known ages.

No attempt was made in this study to examine buried teeth for bacteria after the period of burial but clearly this would be an interesting study. Recognizing the possibility of an effect caused by bacteria only caries free material was used in the experiments.

The second , and most important , objective of this study has been achieved , namely an investigation into the effects of "prolonged" burial in the desert and the influence this may have upon the rate of continued racemization of aspartic acid in dentine.

In summary of the racemization experiments I have established in Kuwait the procedures for estimating age of a deceased from dentine using knowledge of the racemization of aspartic acid and I have discovered that while we can carry out this procedure on fresh material with an accuracy of +/- 1.2 years interpretation of the findings from buried teeth is difficult. I have shown that the temperature of desert soil at 0.5M and 2.0M is remarkably comparable and for five months in the year

approximates to body temperature. This has important implications for estimating age since the aging process may continue at almost "live" rates.

However the results show that the relationship to temperature is not a simple linear one and that other factors such as humidity , pH and bacterial contamination may have a bearing upon results. Clearly further investigations are required but from a practical point of view it would appear that **teeth left in the desert will continue to age significantly and that estimates of age based on racemization must take this into account.** A knowledge of how long a body may have lain in the ground will be important and further longer studies are now required. Work to examine burial sites in other countries is now required in order to further determine the effects of humidity and pH as well as temperature. The invasion of buried calcified tissue by bacteria , their identification and effects also requires much more work.

5.9Age determination from trace elements (Zn, Pb, Fe, Mn, Mg, Sr, and Cu) in Kuwait.

It has been suggested , as discussed in the Literature Review that the trace element composition of dentine shows a correlation with age (41). A possible advantage of using the trace elements level as a tool in forensic studies in the Gulf Region is that not only may different populations have a different "base line" depending upon the geographical location (42) but the expertise and technology for trace element quantitative studies (especially heavy metals) is readily available within the oil companies. These companies have scientists working for them who are trained geologists and analytical chemists. It was for this reason that I attempted to explore the possibility of using quantitative trace element analysis of teeth to establish age at the start of my work and hence the numbers of teeth used for this work are greater. However, as discussed below , it became apparent that the method was not accurate and I began to work on racemization techniques as a preferred method. The two years it took to establish the racemization technique had severe implications for the numbers of teeth I could process this way. Also their preparation for racemization

analysis is much more time consuming and exacting than preparing teeth for trace element analysis

5.10.1 Age determination from trace element analysis of control teeth (not buried).

Although the amounts needed to meet human nutritional requirements are often very small, essential trace elements are necessary for efficient energy metabolism, and for other functions, such as cognition, immunity, and reproduction. Except for iodine, (I), and iron (Fe), for which the essentiality for humans was established more than a century ago, the role of some of the other elements, such as zinc, (Zn) and selenium, (Se), in humans has been recognized only during the last few decades (103 , 104). Deficiency in the intake, or reduced absorption from the gut of trace elements has serious consequences for mental and physical functions. Fe deficiency anemia and goiter due to I deficiency, and probably growth retardation due to Zn deficiency, are examples illustrating the impact of trace element deficiencies. Learning disabilities, impaired work capacity and in extreme cases, death have also been attributed to trace element deficiency (105-107).

Population explosion, poverty, illiteracy, and environmental pollution are common in most developing countries. Only 5-10% of the population in most developing countries can be compared to that of affluent countries with regard to the general standard of living. Although starvation and malnutrition are restricted to certain poverty-stricken areas of the world, marginal deficiency of micronutrients, including that of trace elements, may be widespread. Deficiency of a few trace elements, such as Zn and Se, has also been observed in the general population of affluent countries (108-112). The absence of characteristic symptoms and the lack of simple and adequate diagnostic techniques are the main reasons that a marginal deficiency of many trace elements is not detected at an early stage.

From a public health point of view, trace element nutrition has attracted only desultory attention in most developing countries. Well conducted studies showing the

impact of trace element deficiencies in developing countries in Asia, Africa, and South America are limited. Data available concerning the trace element status of the general population are also very sparse in most developing countries. Further, trace element supplements in vulnerable groups exist only for a few elements, such as iron (Fe) and iodine, I (113).

Similar to Fe deficiency , mild to moderate deficiency of Zn is very common in many parts of the world. Low dietary intake and gastrointestinal disease that may limit the uptake of Zn from the gut are the major causes of Fe and Zn deficiency in developing countries. Information about the daily dietary intake of trace elements based on sound techniques is scanty in many developing countries. The intake data reported is often higher than the true intake because of inadequate methods used for calculations (112 , 114 , 115) but the health authorities depend on the available data when making recommendations for the daily intake of trace elements. During the last few decades public health authorities in different parts of the world have started to take an active interest in defining desirable levels of trace element intake for their populations. Some of these efforts have been duplicated at the international level by bodies such as the World Health Organization (WHO), and the Food and Agricultural Organization (FAO) who are currently involved in a number of international projects dealing with nutritional aspects of trace elements in health and disease (116 , 117). Recently a global database on the human dietary intake of trace elements was published (118 , 119).

For age determination from trace elements in teeth, for a Kuwaiti population, the relationships between age and each trace element [Zn, Pb, Mn, Fe, Sr, Mg and Cu] was calculated based on regression analysis using mean values as the basis of the comparison. Because the variables were continuous, multiple linear regression analysis was suitable for this purpose [120].

Multiple linear regression is concerned with providing a mathematical model of the linear relationship between a dependent variable (ie age) and two or more independent variables (Zn , Pb, Mn , Fe , Sr , Mg , Cu). In particular the mean level

of a dependent variable may be predicted from a set of values for the independent variables.

Multiple linear regression analysis programs are available in many statistical packages such as Minitab and SPSS (Statistical Package for Social Sciences). We used the SPSS in developing the model used in the following analysis.

There were 300 different Kuwaiti teeth not buried in soil (140 Males and 160 Females).
The calculated result for the total 300 Kuwaiti teeth was :
R square = 60.0%.
The SE (standard error for estimation) investigated by descriptive analysis was = ± 5.3 years.

For Males the result was:
R square = 56.7%
SE = ± 5.47%

For Females the result was:
R square = 63.1%
SE = ± 5.4 years.

Therefore in the present study we found that the standard errors were high and there was no significant difference between male and female results.

Early studies (121-125) described differences in the concentration of Zn in teeth, (enamel and dentine), in different geographical areas. In this study of the relationship between trace elements and age, we found that for Zn there was a significant correlation difference ($P < 0.01$) with age in both male and female.
Similar results were described by Derise et al in 1974 (126) and Lappalainen and Knuuttila in 1979 (125) who demonstrated the accumulation of Zn in permanent human teeth with age..

Lead accumulates in teeth. The more polluted the environment the higher the level and in many countries exfoliated deciduous teeth have been used to determine the level of environmental lead pollution. (122 – 130).

Stringer et. al.,1974 (131) found significant variations in lead concentration in lungs with age. In dense bones the correlation of lead accumulation and age is controversial. Some have found an association (132-134), whereas other (135) found no correlation with age. In deciduous teeth, the lead concentration increases with the child's age. The same has been demonstrated in the permanent teeth of adults' (136), although the number of samples was small. Each sample, however, consisted of several teeth. Further, no adequate statistical tests were applied to verify the significance of the age differences in lead levels in teeth. Later Al-Naimi, Edmonds and Fremlin 1980 (137) showed the same result, but analyzed only a very small volume of each whole tooth. Frank et al, 1990 (138) found significantly higher lead levels in the dentine of young subjects (age 10 – 29 yr.) than in older ones (age 32 – 65 yr.), but did not look at smaller age-range groups.

In our experiments we found a significant association between lead levels and age ($P< 0.001$). The regression equation for 300 teeth was :

$T = 2.561 + 0.385 * LnPb$

Estimated age = EXP(T).

The correlation coefficient (R square) = 52.3 % and the standard errors for estimation (SE) = ±5.8 years.

In a similar study (125) Lappalainen and Knuuttila also claim that lead concentration increases with age but the regression coefficient they recorded was 0.37 with R square = 0.44%

Clearly the uptake of lead is very variable and will depend up such variables as the proximity of children to industrial complexes releasing large quantities of lead into the atmosphere , lead in drinking water and fumes from car exhausts where leaded petrol is still used. The accumulation of lead with age is therefore more useful as a

marker of pollution rather than a method of estimating age . Teeth are a particularly good marker of lead pollution since lead accumulated in enamel during enamel formation is not lost and modern analytical methods can distinguish different isotopic forms of lead which in turn identify the source as either (for example) lead water pipes or petrol.

No significant differences were found between the lead levels in teeth from males and females in Kuwait. Both sexes were exposed to the same environment pollution, as we assume there is no sex difference in the anatomy of the gastrointestinal tract or of lungs, absorption should be the same in both males and females. Other workers have published similar findings.(41,134,139).

5.10.2 Age determination from trace element analysis of teeth buried at depths of 0.5M and 2.0M.

The impact of heavy metals on biological systems has been widely studied [140-142] but there are few studies establishing a definite correlation between environmental quality and the state of health of the people [143-145].

Many human tissues, (hair, bone, teeth, and nails) can be used as biomarkers of the environmental burden of toxic metals [143,146-150].

The bioaccumulation of heavy metals in human hair and teeth is rather a complex process. Factors that influence bioaccumulation include: nourishment, chemical forms of the metal and their binding site (151) as well as age, sex, genetic inheritance, and environmental quality [146].

The mechanism of the interrelationships between heavy metals in human organs and age are still being studied. The changes in the metal ratios for different age groups are certainly dependent on the metal homeostasis in human organs and they can be used as indicators of the physiological processes occurring in the living systems [152].

This work attempts to establish the age of teeth buried at depths of 0.5M and 2.0M by determining the effect of burial on the measurement of trace element [Zn,Pb,Fe,Mg,Sr,Mn and Cu] . Bone Pb levels (tibia and patella) had a strong positive correlation with age [153], and in teeth, we can observe an increase in Pb levels with age, too [154,155].

The R square for 19 third molar teeth buried in the soil at a depth of 0.5M= 49.2%
The SE (standard error of estimation) was investigated by descriptive analysis between known age and calculated age.
SE = ± 3.0 years.
The R square 16 third molar teeth buried in the soil at a depth of 2.0M= 45.5%
The SE (standard error of estimation) was investigated by descriptive analysis between known age and calculated age.
SE = ± 3.3 years.

It is apparent that while there is a correlation between age and trace element levels it is poor compared to the association between age and racemization

Interestingly it was found that, the standard errors at both depths was high but less than in teeth not buried (control). This difference in standard errors may be due to absorption of trace elements from the soil and/or loss of trace elements into the soil resulting in a degree of "uniformity" in trace element concentration between samples exposed to the same local environment. This suggestion requires to be tested.

In both males and females it was found that Zn, Mn, Ph, and Fe concentration was lower in the dentine of teeth buried at both 0.5M and 2.0 M than in teeth not buried while Sr and Cu showed higher concentrations in teeth buried at depth 0.5M and 2.0M than in teeth not buried.

The relationship of the trace element concentration to the crystallographic data of biological apatites has been studied by LeGeros et al.1977 [156 , 157]. The effects of the trace elements may be connected with the absorption of ions on the surface of

lattices or with the ionic exchange phenomena [158 ,159]. The cationic trace elements might have coordinate links with the phosphate tetrahedron or other substituents of PO groups such as CO . the interactions can be based on diadocia as well as on isotypism. Both possibilities are usual in naturally occurring apatites [160].

Many different cationic elements have been connected with mineralization [161 ,162 ,163 ,164]. Trace elements can produce crystallographic change under in vitro conditions [165].

The interactions between the cationic elements are significant because a balance should be formed with respect to the ionic size, the coordination and the chemical properties of ions in order to obtain a chemical balance. Although many relationships between cations have been described in non mineralized tissues, the situation in bone, dentine or enamel is obscure. Cu-Zn. Sr-Ni and Cu-Mn interactions in mineralized tissues have been studied [157 , 166-168].

Further studies are, however, needed to evaluate possible causal relationship and explaining the influence of environment in teeth buried in ground containing heavy metals.

This study was carried out to determine whether is any changes in the level of trace elements (Zn, Pb, Mn, Fe, Sr, Mg and Cu) in the dentine of the teeth buried at depth 0.5m and 2.0m as compared to the teeth that were not buried, in males and females, respectively. Also a comparison was made in the level of trace elements (Zn, Pb, Mn, Fe, Sr, Mg and Cu) between the teeth of males and females that buried in sand soil at different depths as mentioned. These studies were done to simulate the environmental conditions of the burial grounds and to understand whether these conditions could have any effect on the level of trace elements present in the dentine.

The overall significance of the models was tested with T-test ; the values of the T-test were statistically significant ($P< 0, 001$).

The results show that there was a significant difference in the level of Zn and Sr in teeth buried at depth at 0.5m and 2.0m in males. The Zn level in dentine showed a

decrease at both depths (compared to teeth not buried) whereas the Sr levels showed an increase. In addition to the changes in the levels of Zn and Sr, the levels of Mn and Cu showed a decrease and increase respectively in the teeth buried at both the depths as compared to the teeth that were not buried. However, while this difference in the level of Zn and Sr compared to teeth not buried was statistically significant at 0.5m depth differences were not significant at 2.0M. Pb showed a decreased concentration at both depths. This decrease was not statistically significant at 0.5m but was significant at 2.0m.

In general Zn, Pb, Mn and Fe showed a decrease in their levels as compared with the teeth were not buried, whereas, Sr, Mg and Cu showed a general increase in their level in the teeth of males following burial. This general trend was followed in the teeth of females too, however, the magnitude of decrease and increase in females was more conspicuous than males.

In females, the decrease in the level of Zn, Pb, Mn and Fe was statistically significant, whereas the changes in the level of Sr, Mg and Cu were not significant at a depth of 0.5m. while the differences in the level of almost all the trace elements in the dentine of the teeth of females, which were buried at depth 2.0m were significant except for that of Pb and Cu.

There were no significant differences in the level of Zn, Mn, Sr, Mg and Cu between the males and females, in the case of the teeth that were not buried. However, significant decrease was observed in the level of Pb and Fe at depth 0.5m and Zn at 2.0m.

Beelay and Lunt 1980, (169) claim that, archaeological dentine is often softened, or brittle and fracturing of underlying dentine causes the break-up of otherwise intact enamel. What effect this may have upon trace element content of both enamel and dentine is not known. Viewed in tooth sections with the naked eye, affected dentine is chalky or opaque and is found especially near the root surface or pulp cavity. In light microscopy of ground sections the damage appears to be due to irregular canals

meandering through the dentine. (170).It is usually assumed that they are made by invading fungal mycelia. The SEM image of affected dentine is, however, often different from the light microscope appearance, and back scattered electron (BSE) imaging demonstrates marked changes in mineralization (171), Clear canals are visible in some specimens, although the dominant disruption is usually scattered foci of diagenetic change some with low densities, but many with higher densities than the intervening unaffected dentine. They tend to follow the incremental lines of intertubular dentine, whereas peritubular dentine is often left intact. Poor structural integrity of ancient dentine appears related to collagen loss, but not all specimens are affected and some even show organic matrix stain reactions. (171-173), Preservation is difficult to predict and there is no consistent relationship with the length of burial time.

Obviously variation in soil chemistry , environmental conditions and the presence of soil flora and fauna (especially certain bacteria) may be responsible for the differences in the level of trace elements observed at the two different depths as compared to the teeth not buried.

It is reasonable to suggest that temperature , humidity and pH could all influence the various properties of hard biological and plastic materials (174 , 175). Similarly the selective uptake of certain trace elements by soil bacteria and other organisms could influence the level of trace elements in dentine. Various studies show that plants and bacteria have the ability for selective uptake and release of various trace elements (176 - 178). Further studies are necessary to determine the effect of diffusion and the leaching of trace elements from dentine in different environmental conditions. Similarly it is necessary to understand how different bacteria can deposit and selectively uptake different trace elements.

To the best of my knowledge studies of this nature, showing the differences in dentine trace element concentrations after the burial of the teeth, have not been carried out . **Therefore the results presented here, showing the differences in the level of trace elements in the males and females of the teeth that were buried as well as the differences obtained in the level of trace elements between males and**

females are unique. Obviously it is necessary to carry out further studies to understand the exact biochemical or biological mechanisms that are responsible for this change in the level of trace elements in the teeth after they have been buried in the soil.

However , interesting as such studies might be , what is apparent from my work is that the variables concerned in trace element analysis and interpretation are so great , and the reliability of the racemization techniques so good as to **confirm the use of trace element concentrations for age estimation as inaccurate and irrelevant.**

5.11 Comparison between D-and L-aspartic acid analysis and trace element analysis

In this study we analyzed one type of tooth. upper first premolar dentine was bisected vertically and one half used for amino acid analysis and the other for trace element analysis. From D- and L-aspartic racemization we found that, the stand error was ±1.2 years and the stand error was ±5.34 years from trace elements analysis. **From this finding we found that D-and L-aspartic acid analysis was much more accurate than trace element analysis.**

The proteins in dentine are metabolically isolated and are held at a constant temperature (37°C ± 1°C) during life (29, 74). Trace elements can, by contrast, produce crystallographic changes (156 ,161 ,179 ,180). The effects of the trace elements may be connected with the absorption of ions on the surface of lattices or with ionic exchange phenomena (181 , 182). The cationic trace elements might have coordinate links with the phosphate tetrahedron or other substitutes of PO_4 groups such as CO_3. The interactions can be based on diadocia as well as on isotypism. Both possibilities are usual in naturally occurring apatites (160).

The interactions between the cationic elements are significant because a balance should be formed with respect to the ionic size, the co-ordination, and the chemical properties of ions in order to obtain a chemical balance.

In this study of relationships between trace elements [Zn,Pb,Fe,Mn,Sr and Cu] in dentine of a Kuwaiti population, we found that there was a positive correlation between Zn and Pb, Mn and Fe- Mg and Cu ($P<0.001$). Lappalainen and Knuuttila in 1982 (162) claimed that, closely related metallic elements such as Co, Cu, Mn and Ni are positively correlated in dentine.

From our findings, I conclude that D-and L-aspartic acid analysis can be related to body temperature but the trace element analysis provides unreliable data since the concentration of elements is so variable from one individual to another and from one population to another.

5.12 Gender determination of teeth not buried and buried in the soil

Of all demographic characteristics, differences between the sexes have probably been studied the most ; almost every human bone has been analyzed in this regard (183 - 185). Apart from its obvious importance in forensic applications, and understanding of sexual dimorphism it is also fundamental to the study of human growth and evolution. Theoretically, this assessment should be easy once puberty is reached and males and females diverge significantly to follow their distinct, genetically determined forms and reproductive functions.

In spite of the obvious differences and numerous studies of sexual dimorphism, sex determination from the skeleton can be problematic in some cases. The definitiveness of differences between fleshed individuals stands in stark contrast to what is seen in many bones. The problem arises from the contradiction between discrete, genetically determined sex classifications and the continuous transitions of somatic sexual characteristics in the phenotype. A simple analysis of sex differences always shows a certain overlapping of the two sexes; in the skeleton sex is not

binary, even though a normal human is either a man or a woman. This diagnosis is also influenced by a number of other factors such as the environment, age differences, pathological changes, and, above all, biological variation. Even various populations of the same race may exhibit different manifestations of sexual dimorphism, which may change with time. Thus, it is difficult to develop a method that would always partition a given population into males and females. These problems are still being investigated (186). The applied aspect was discussed at the Workshop of European Anthropologists (187).

The pelvis and skull are excellent sites for sexing and it is important to make the most of both. Although sexual dimorphism is better expressed in the pelvis, this site is very often damaged. The skull, on the other hand, is usually better preserved, but its sexual differences are not quite as definitive. A long-standing argument also centers on whether morphological (i.e., visual) or morphometric traits are more effective (188). The evaluation of morphological traits is thought to be more subjective and depends on the experience of the investigator.

The complex pattern of male/female differences in tooth crown diameters through the dentition suggests that a multivariate approach might form the basis of a method for sex determination. In studies, 60-90% of children have been correctly assigned to male or female from their deciduous crown diameters (189 , 190). Ditch and Rose, 1972 [191] and Rosing, 1983 [192] tested dental discrimination in archaeological material, sexed independently from a study of the pelvis, and obtained correct classifications in 90% of cases. Similar methods have potential for studies of fossil hominids. Bermudez de Castro and colleagues,1993 [193] used a reference group of archaeological material from Gran Canaria Island to classify teeth from the Middle Pleistocene site of Sima de los Huesos (Sierra de Atapuerca, Spain) and the Eastern European Neanderthal group from Krapina in Croatia. Using a single collection of archaeological material, sexed by skeletal features, to classify much more ancient and completely unrelated material is fraught with difficulty even though the Krapina material does show two quite clearly separated groups in tooth size. In a large collection of collection of archaeological material, it would be better to establish a

baseline group, in which the sex was established by reliable indicators in the pelvis, and to base classification of unknown specimens on the pattern of crown diameters seen in this groups. It must also be remembered that the actual size differences between sexes in individual teeth are very small (about 0.4mm to 0.5mm), so that observer error may well be an important factor (194).

In this study we examined the racemization analysis and trace element analysis by multiple discriminate analysis for gender determination in a Kuwaiti population.
Using the racemization data it is possible to ascribe correctly 69% of the male teeth to the male category and thus show a difference between male and female teeth with regard to racemization. This difference was significant ($p< 0.001$). Burying teeth made no significant difference to this finding. The corresponding % figure for burial at both 0.5M and 2.0M being 66.7%.

I do not know why it is possible to ascribe 69% of the teeth accurately in this manner. However Carney (195), reported that; the sex hormones cause disintegration of ground substance; progesterone, in particular, affects the microvascular system of the gingiva, making the gingiva more suspectable to irritation. The estrogen causes edema of the sexual organs as well as other parts of the body and an increased production of mucopolysaccharide (196 – 197).

Estrogens are known to exert an effect on bacterial infections. Thus, streptococcal infections are partly inhibited by estrogens (198-200). The action of estrogen may be mediated by for example an increased concentration of immunoglobulin (201), or by a greater phagocytic capacity of the monocytic system (199).

Oral bacteria are known to produce various toxins and enzymes capable or causing tissue destruction (202 , 203). Dental plaque, itself, is composed of various bacteria, leukocytes, macrophages and desquamated epithelial cells bound within a mucinous matrix in close proximity to the sulcular epithelium.

Most of the organic component of dentine, cement and bone is protein. Survival of proteins in buried bones and teeth is highly variable [204].

Collagen is very stable, but can be broken down by boiling in dilute acid to produce gelatins, which are composed of short lengths (peptides) of a-chain. Collagen is also broken down into peptides in buried specimens and the peptides further broken down into their component amino acids, which may then be leached from the specimen by percolating groundwater. The extent to which all this actually happens varies, even within one specimen, and does not necessarily reflect the surface appearance of preservation. In dentine with 11% protein surviving, the proportions of different amino acid residues any differ little from those of fresh dentine, whereas they diverge substantially in specimens with less then 1 % protein (169). The chemical basis of collagen breakdown in the soil is in any case not well understood, but is dependent upon temperature, groundwater and its pH. Microorganisms must be involved and are inhibited by cold, dry, or waterlogged conditions.

Given the effect of estrogens on bacterial infection it is possible that the gender difference discernable in the racemization study is mediated via bacteria. At this stage this is just speculation but a real gender difference does appear to exist although insufficiently reliable to be used to definitely determine whether a single tooth was male or female.

Gender determination from [Zn,Pb,Mn,Fe,Sr,Mg, and Cu] from teeth not buried and buried at depth 0.5m and 2.0m by using multiple discriminate analysis was also performed.

Curiously the result was better than that aquired from the racemization study with 80% of the teeth being correctly ascribed with regard to gender. The result is curious because of the multiple environmental variables to which the teeth were exposed. Gender differences related to diet and habits such as smoking might need to be considered.

It must be admitted that this part of the work was only carried out because suitable data was available. I , personally , do not think it is worth persuing further because since I began this work DNA technology has advanced considerably and genetic

techniques for determining sex accurately from teeth are likely to be a much more fruitful field of research

5.13 Future study and potential for forensic applications

Estimation of age from dentine by using the High Preformance Liquid Chromatography (HPLC) technique for the separation of D- and L-forms of aspartic acid is highly sensitive enough for practical use.
The affect upon racemization of aspartic acid in teeth buried in the desert of the Gulf States at a depth 0.5M and 2.0M over 10 months is clearly important. Racemization continues in buried teeth due to prolonged relatively high temperatures, high pH and low humidity. These finding are important since the estimation of age of an unknown body in similar conditions in the future must new take notice of temperature, pH and humidity and all should be measured at the burial site.

Now that this basic information has been elicited experiments should be designed to study the changes over a longer period , in different soils and in different climates. A multicentre study with teeth buried in very different parts of the world would help establish a data bank and from the results enable , in time , information to be gathered that would enable investigators to scientifically take into consideration the effect of temperature , pH , humidity of the soil and possibly the effect of bacteria when calculating the age at death of an unknown body. Clearly the problem is that to do this there must be information from another independent source to suggest how long the body has been buried. Where that information is not available the findings described in this Thesis strongly suggest that age estimation will not be as accurate as the literature up to now has suggested.

Controlled laboratory experiments to study the isolated effect of bacteria (which bacteria ? and in what concentration ?) upon racemization might be revealing.

Analysis of ancient DNA in bone and teeth can potentially provide archaeological information on human population movements, kinship and sexing [205 , 206]. Initial studies [207 , 208] found that cloning the highly damaged DNA was very difficult and so it was assumed that limited sequence information could be obtained from only exceptionally well preserved specimens. However, the advent of the Polymerase Chain Reaction (PCR) allows direct amplification of specific DNA fragments from minute amounts to quantities suitable for sequencing without the need for cloning. [209 - 211]

Since 1990, it has been shown that a broad range of archaeological remains contain preserved DNA and the application of advancing new techniques is pushing back the age of DNA that can be extracted. However, reports of the extraction of several million years-old DNA have been challenged by estimations of the rate of chemical decay of the DNA structure [181 ,182 ,212].

One approach in the study of DNA diagenesis is to try to relate the survival of DNA to protein preservation in bone on which much more research has been based. The complexity and cost of DNA analysis have resulted in the development of screening methods which may indicate good preservation, thus saving the analyst fruitless attempts at amplification. All these screening methods, such as histological indices [213 ,214] nitrogen [215] and aspartic acid racemization [216] relate either directly or indirectly to protein diagenesis.

In future studies we need to investigate the survival of DNA protein in teeth buried in the desert conditions of Kuwait . The pulps of teeth may prove to be very useful because they are isolated from the effects of the soil and other possible contamination.

Chapter 6

CONCLUSIONS

Estimation of age at death from dentine from upper first premolar teeth in Kuwait using the High Performance Liquid Chromatography (HPLC) technique can be used for the separation of D- and L- forms of aspartic acid in minute amounts of dentine, and the sensitivity is high enough for practical use. This method was shown to be accurate to ± 1.2 years.

From this it is concluded that the method provides good results but more teeth from older persons are required to establish a reliable base line.

The affect upon racemization of aspartic acid in teeth buried at depths of 0.5M and 2.0M over a 10 month period was studied. Racemization continued in the buried teeth but the results were less constant than in the not buried teeth with a SE = ±2.3 years in the teeth buried at a depth 0.5M and ±3.0 years at 2.0M. The D/L ratio was significantly different between buried teeth and not buried teeth.

The results demonstrated continued post mortem racemization in the warm soil and the modification of the rate of racemization has been discussed in the context of prolonged exposure to temperatures near to body temperature , relatively high pH and low humidity.

These finding are important since estimation of age of unknown body in the desert conditions of the middle east must now take notice of temperature, pH and humidity, all of which should be measured at the burial side.

It is evident from this work that any age estimation by racemization from teeth buried in the desert will be the upper age limit and the victims actual age may several years younger depending upon how long the teeth have been buried. The age difference therefore between estimated age and actual age will be different not only because of pH, humidity and temperature but from the length of time the teeth have been buried in these conditions.

It is new apparent from these results that teeth of known age require to be buried for much longer periods in conditions for which the pH, humidity, and temperature are known in order that reliable data concerning the effect of these variables upon racemization may be more accurately established. When this data has been acquired it may be possible to accurately determine the age at death of buried skeletons in most parts of the world.

What is clear from the results describe in this thesis that without knowledge of pH, humidity, temperature and length of time that the teeth have been buried, accurate estimation of age is not possible.

Formaldehyde storage is not recommended as fixation cross-links proteins. The best storage method is to keep the teeth dry at a low temperature
(-70°C).

Methods of washing should be consistant as different washing substances remove different species of proteins from the dentine.

From the findings described in this Thesis it was confirmed that to obtain accuracy in age determination from teeth measuring the racemization of aspartic acid in dentine is much to be preferred to trace element analysis because body tissues are held at a constant temperature (37°C) with a constant rate of racemization. Trace element concentrations are very variable and although there was demonstrated an accumulation of most elements with age they were not constant from individual to another and the variables to be considered were very numerous. The method proved to be inaccurate , time consuming and eventually redundant since the results using racemization proved to be both accurate and reliable.

However trace element analysis could still play a useful role in forensic science on occasions. Accumulation of heavy metals such as lead in enamel provide a historical record of pollution and may help to determine or at least suggest where the deceased lived during life and this may provide a clue as to his identity.

Although using both racemization and trace element methods it was possible to indicate a probability of gender in any tooth examined the degree of accuracy is poor and it is anticipated that DNA analysis will be much more useful. The future role of tooth pulps in DNA identification is briefly discussed.

Chapter 7

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